Global Approaches to Cocoa Germplasm Utilization and Conservation

Final report of the CFC/ICCO/IPGRI project on "Cocoa Germplasm Utilization and Conservation: a Global Approach" (1998–2004)

A.B. Eskes and Y. Efron, editors

IPGRI

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[1] Project Coordinator
IPGRI/CIRAD
c/o INIBAP
Parc Scientifique
Agropolis II
34397 Montpellier
Cedex 5
France

[2] Former Project Coordinator at CCI
Deganiot 48
Tivon 36054
Israel

CFC Technical Paper No. 50

The **Common Fund for Commodities** (CFC) is an inter-governmental financial institution established within the framework of the United Nations. The Fund's specific mandate is to support developing countries that are commodity-dependent to improve and diversify commodities' production and trade. The Common Fund finances projects that typically have a market development and poverty alleviation orientation. Its multi-country dimension and approach places a high premium on the development of regional commodities-based economies, through a variety of project initiatives that seek to enhance the capacity of commodity producers and exporters to participate fully in global trade. By the end of year 2005, the Fund had a full portfolio of 224 development initiatives valued at US$ 412.8 million, with 85 percent of the projects financed through grants. The Fund currently has a membership of 106 countries. Institutional members include the European Community (EC), the African Union (AU) and the Common Market for Eastern and Southern Africa (COMESA).

The **International Cocoa Organization (ICCO)** was established in 1973 under the auspices of the United Nations to administer the provisions of the International Cocoa Agreement (ICA), 1972 and its successor Agreements of 1975, 1980, 1986, 1993 and 2001. The Agreements were concluded among the Governments of cocoa-producing and cocoa-consuming countries at conferences convened by the United Nations. In March 2001, the Sixth International Cocoa Agreement was concluded in Geneva and came into force in October 2003.
The International Cocoa Agreement, 2001, entrusted the ICCO with an explicit mandate to achieve a "sustainable world cocoa economy". For the ICCO, "sustainability" comprises dimensions of an economic, environmental and social nature, related to production and consumption of cocoa and chocolate. The ICA, 2001, also established a Consultative Board on the World Cocoa Economy consisting exclusively of experts from the private sector, in equal numbers from cocoa-producing and cocoa-consuming countries.

The three priority areas for implementation of the current International Cocoa Agreement are: Cocoa prices, farmers' incomes and export revenues; Market Access, Market Information and Market Development; and Sustainable Cocoa Production.

The **International Plant Genetic Resources Institute (IPGRI)** is an independent international scientific organization that seeks to improve the well-being of present and future generations of people by enhancing conservation and the deployment of agricultural biodiversity on farms and in forests. It is one of 15 centres supported by the Consultative Group on International Agricultural Research (CGIAR), an association of public and private members who support efforts to mobilize cutting-edge science to reduce hunger and poverty, improve human nutrition and health, and protect the environment. IPGRI has its headquarters in Maccarese, near Rome, Italy, with offices in more than 20 other countries worldwide. The Institute operates through four programmes: Diversity for Livelihoods, Understanding and Managing Biodiversity, Global Partnerships, and Commodities for Livelihoods.

With effect from 1 December 2006, IPGRI and INIBAP will operate under the name "Bioversity International", Bioversity for short. This new name echoes their new strategy, which focuses on improving people's lives through biodiversity research.

The geographical designations employed and the presentation of material in this publication do not imply the expression of any opinion whatsoever on the part of CFC, ICCO or IPGRI concerning the legal status of any country, territory, city or area or its authorities, or concerning the delimitation of its frontiers or boundaries. Similarly, the views expressed are those of the authors and do not necessarily reflect the views of these organizations.

Citation: Eskes, A.B. and Y. Efron, editors. 2006. Global Approaches to Cocoa Germplasm Utilization and Conservation. Final report of the CFC/ICCO/IPGRI project on "Cocoa Germplasm Utilization and Conservation: a Global Approach" (1998-2004). CFC, Amsterdam, The Netherlands/ICCO, London, UK/IPGRI, Rome, Italy.

Cover illustrations: Flower of *Theobraoma cacao* (D. Clément, CIRAD), and different stages of clonal (A.B. Eskes, IPGRI/CIRAD) and hybrid (D. Paulin, CIRAD) cocoa trees.

ISBN-13: 978-92-9043-734-5
ISBN-10: 92-9043-734-0

PREFACE

Cocoa is a commodity produced in the developing countries of the tropics and consumed mostly in the middle- and high-income countries of the world's temperate zones. Currently, over 50 countries engage in cocoa production, of which some heavily rely on cocoa exports for their economic development as they contribute significantly to their foreign exchange earnings.

From a level of 1.5 million tonnes in 1983-84, world production of cocoa beans is steadily rising and has reached a peak of 3.5 million tonnes in 2003-04. This significant increase is almost entirely due to an expansion of production area. Over 90% of world cocoa is produced by smallholder farmers who rely almost entirely on the supply of improved planting material from national and international research institutes. Nearly all producing countries grow cocoa on an extensive basis resulting in low average yields, which – on global average – have only increased little over the past three decades. This contrasts with the often dramatic advances in yields of other tropical or temperate crops and in particular for other raw materials, often used to manufacture snack foods which are competitive with cocoa. Gains in global yield and productivity of cocoa are now essential. As pressure on available land increases, the need for higher yielding, pest- and disease-resistant cocoa varieties becomes ever more urgent.

This Technical Paper is the result of work undertaken in the CFC/ICCO/IPGRI project: *"Cocoa Germplasm Utilization and Conservation: a Global Approach"*, which aimed at a more sustainable production of cocoa at lower costs, by making optimal use of cocoa germplasm. Special attention was paid to the evaluation and selection of resistance to some of the major diseases and pests, such as black pod, witches' broom, vascular streak dieback, moniliasis, cocoa swollen shoot virus and mirids, which together cause losses of an estimated 40% percent of annual world cocoa production.

The Common Fund for Commodities acknowledges the significant inputs of both the International Cocoa Organization (ICCO) as Project Supervisory Body, and the International Plant Genetic Resources Institute (IPGRI) as Project Executing Agency for the successful implementation of the project in 12 countries. In line with the policy to disseminate the information produced by activities financed by the Fund, it is my expectation that this publication will be instrumental to make the results and experiences of this project available to a wider audience. It is hoped that extension workers, researchers and policy-makers would find this publication useful and relevant for improving access of higher yielding, good bean quality and disease-resistant cocoa varieties to farmers.

Amb. Ali Mchumo
Managing Director
Common Fund for Commodities

ACKNOWLEDGEMENTS

The advances made in the CFC/ICCO/IPGRI Project, as summarized in this report, are to be credited to the scientists and support staff of the thirteen collaborating institutions involved (for the full list see Appendix II). Especially, the Technical Coordinators of these institutions are to be acknowledged: Jeanne N'Goran (CNRA), David Iwaro (CRU), Didier Paulin (CIRAD-CP), Freddy Amores (INIAP), Kelvin Lamin (MCB), Kolawole Badaru and Peter Aikpokpodion (CRIN), Paul Hadley (University of Reading), Salomon Nyassé (IRAD), Ventura Gonzalez (FUNDACITE), Vish Mooledhar and Kamaldeo Maharaj (MALMR), Wilson Monteiro (CEPLAC), Yaw Adu-Ampomah (CRIG) and Yoel Efron (CCI).

Special thanks go also to the Directors and Administrative Project Coordinators of all collaborating institutions for their invaluable support to administrative aspects and implementation of the activities.

The CFC/ICCO/IPGRI Project was developed under the aegis of the International Cocoa Organization and mainly funded by the Common Fund for Commodities. However, the implementation of the project would not have been possible without the significant counterpart and co-financing contributions of all collaborating institutions in the Project. The Common Fund for Commodities and the co-financing institutions (ACRI – now WCF –, BCCCA, CIRAD and IPGRI) are acknowledged for their cash and in-kind contributions, and the national research institutions for their in-kind contributions and dedication to the project activities. ICCO is acknowledged for its important role during the establishment phase of the project and for its continued supervision and general support received during the implementation of the project.

We wish to thank particularly:

- Jan Engels, for his important role in the setting-up of the project, for his continuous technical support, critical reading of project publications and active participation in project meetings;
- Tony Lass, for his important role in the setting-up phase of the project and for his steady support to the project as the Chairman of the "Co-financiers Working Group", during the implementation of the project;
- Emile Frison and Richard Markham, for their administrative support to the Project Coordination Unit;
- Bernadette Sellers, Gillian Moffatt and Anne Causse, for their kind and excellent services as the successive Programme Assistants of the Project Coordination Unit;
- Tom Thornton and Emmanuel Gonnord, for their contributions as Financial Officers of the Project Coordination Unit, and
- Elinor Lipman, for the style editing and layout and for the stimulating support received in finalizing the manuscripts.

The editors

Bertus Eskes
Yoel Efron

CONTENTS

INTRODUCTION

This Final Report summarizes the outcome of the Closing Workshop of the CFC/ICCO/IPGRI project on "*Cocoa Germplasm Utilization and Conservation: a Global Approach*", convened at the end of March 2004. The workshop formally marked the conclusion of a project that was launched in February 1998, but for which the preparations began nearly five years earlier. From the first discussions on the development of a proposal for the "*Conservation and Use of Cacao Genetic Resources*", as the project was initially called, to the submission of a formal proposal to the Common Fund for Commodities took almost three years and from the submission of the proposal to the Launching Workshop in 1998 took a further two years. This illustrates that considerable time and effort were required to achieve consensus between diverse project partners, namely, the interested national cacao research institutes, advanced research institutes and the coordinating and supervisory bodies on the one hand and the donors on the other. We are confident that this Final Report will demonstrate that the long gestation period of the project was fully justified in that it allowed the partners to produce a solid foundation on which the work could eventually thrive!

Cocoa breeding during the 1990s suffered seriously from the low price of cocoa. Breeding programmes were under-funded and therefore had to reduce their activities or even, in some cases, cease their activities completely. When the project started, cocoa breeding had virtually come to a halt in five out of the ten countries that participated in the project. Meanwhile, destructive pests and diseases had invaded new cocoa growing areas, such as witches' broom in Brazil (Bahia), moniliasis in Central and South America, *Phytophthora megakarya* in West Africa and pod borer in Indonesia.

Most of the genetic diversity of cocoa is found in the Americas, whereas most of the cocoa crop is produced in Africa. Links between national collections and the main international cocoa collections were weak, and much of the cocoa germplasm had not been properly characterized and evaluated. Furthermore, the links between breeding and conservation programmes were generally weak or not existent. As a result, very few of the collaborating cocoa breeding programmes had access to adequate diversity to serve as platform for their efforts. In addition, many breeders lacked adequate training and frequently operated under rather isolated conditions. In sum, international cooperation in cocoa breeding was sorely needed. The project proposed to enhance collaborative cocoa evaluation, including screening for disease resistance, and selection activities, while strengthening national breeding programmes at the same time.

The main actions of the project included:

- Establishment of an International Clone Trial, aiming at distribution of valuable germplasm and evaluation of the stability of cocoa traits worldwide;
- Establishment of Local Clone and Hybrid Trials, aiming at selection of new cocoa varieties;
- Reinforcement of population breeding approaches, aiming at continuous progress in breeding;
- Establishment of a germplasm enhancement programme for *Phytophthora* pod rot resistance, making use of the large diversity available in the Trinidad collection;
- Application and validation, where needed, of early resistance screening methods;

- Identification of a working collection, aiming at making available to breeders a large representation of genetic diversity containing useful agronomic traits; and
- Information exchange and publication of results.

Based on the above-mentioned justifications and actions, the project clearly focused its attention on building capacity among the partners, by linking the research institutes and researchers with one another, by technical backstopping provided through the yearly visits of the project coordinator to all the collaborating institutes, and through the organization of two project workshops and regional project meetings.

Between 1998 and 2004, the project was able to produce very tangible results, including:

- The creation of an effective informal research network;
- The revitalization of cacao breeding programmes in many of the participating countries;
- The implementation of collaborative approaches to cocoa breeding;
- The establishment of approximately 90 ha of new variety trials;
- The indirect support to germplasm collections by applying the concept of "conservation through use";
- The identification of the "Project Collection", combining genetic diversity and agronomic interest;
- The agreement on 31 standardized working procedures to evaluate germplasm for agronomic important traits; and
- The building of extra human capacity.

The results of the project activities were communicated in detail during the Closing Workshop of the project (see programme of the workshop, Appendix I) and were described also in the Final Individual Institute Reports. The presentations made during the workshop as well as the Final Individual Institute Reports were included on a CD-ROM that was distributed to all interested parties in July 2004. However, part of the results were still preliminary, because cocoa varieties need to be observed for at least five years in the field before selections can be made efficiently. The current publication contains those results of project activities that were sufficiently advanced to have a wider scientific bearing and to be of interest to a broad public.

Based on the positive experiences during the project, the existing partners and some new ones were very keen to extend their collaboration to the CFC/ICCO/IPGRI project on "*Cocoa Productivity and Quality Improvement: a Participatory Approach*" that started in 2004. This allowed the momentum to be maintained, extended the collaboration into new areas and began to exploit the promising materials identified in the first project, this time with direct participation of the farmers. IPGRI wishes to thank the donors for making this possible!

J.M.M Engels (IPGRI)
A.B. Eskes (IPGRI/CIRAD)
R. Markham (IPGRI/INIBAP)

PROJECT JUSTIFICATION, OBJECTIVES, STRUCTURE AND EXPECTED OUTPUTS

A.B. Eskes

IPGRI/CIRAD, c/o INIBAP, Parc Scientifique Agropolis II, 34397 Montpellier cedex 5, France

Summary of project proposal

The project was set up with the aim of obtaining more sustainable production of cocoa at lower cost by making better use of cocoa germplasm. An internationally coordinated effort was proposed for the screening, selection and breeding of improved cocoa genotypes. Selection for resistance to some of the major diseases and pests, which are important destabilizing factors in many producing countries, was a priority objective. The project focused on establishing functional links between germplasm conservation, evaluation and selection activities in a joint effort to increase the genetic potential of cocoa germplasm. The project should contribute over the medium and long term to improving significantly the quality of planting material available to cocoa growers.

The following outputs were planned:

- Multilocational clone and hybrid trials, containing introduced and locally available genotypes, established at ten different sites with a view to selecting superior genotypes, assessing their breeding value as well as their genetic stability for economically important traits;
- Population breeding activities initiated or reinforced at four sites, aiming at sustainable improvement of cocoa;
- Internationally conserved germplasm characterized and evaluated to strengthen germplasm enhancement programmes; and
- Selected genotypes from the international collections distributed to interested user countries.

Project Collaborating Institutions were cocoa research institutes in ten cocoa-producing countries (see below) and three international research institutions (CIRAD, CRU and the University of Reading). Co-financiers in the project were British and American chocolate and biscuit manufacturing associations (BCCCA and WCF – formerly ACRI), CIRAD and IPGRI.

It was envisaged that the project would require efficient coordination, training and scientific/technical backstopping. Two workshops were planned, one at the beginning of the project (1998), to decide on working procedures to be applied in the project, and one at the end of the project, to analyze and disseminate results. During the first project workshop it was proposed that mid-term regional technical meetings should be organized in Asia, Africa and America.

The proposed conservation and characterization activities would be supported through counterpart contributions and co-financing, especially by associations of cocoa processors and chocolate manufacturers as well as by CIRAD. Selection, breeding and project coordination depended largely on CFC financing, on counterpart contributions (national institutions) and, to a lesser extent, on co-financing. To achieve success the project would require excellent liaison between all participants.

In view of the long-term nature of cocoa breeding it was anticipated from the beginning that a further phase would be required to consolidate the achievements of the first project.

Introduction

The objective of this paper is to recall the project justification, rationale and organizational structure, the collaborating institutions and the main activities proposed. A short overview is also presented on the resources originally allocated to the project and effectively used.

Project justification and rationale

Average worldwide cocoa yields are about 400 kg/ha, varying between countries from 150 to about 800 kg/ha. The main production constraints of cocoa are diseases and pests. The most important diseases are *Phytophthora* pod rot (Ppr), witches' broom (caused by *Crinipellis perniciosa*, now *Moniliophthora perniciosa*), vascular streak dieback (VSD) caused by *Oncobasidium theobromae*, monilia (*Moniliophthora roreri*) and the cocoa swollen shoot virus (CSSV). The most important pests are cocoa mirids in Africa (*Sahlbergella singularis* and *Distantiella theobromae*) and the cocoa pod borer (CPB, *Conopomorpha cramerella*) in Asia. Together, diseases and pests are responsible for about 35% losses in production of cocoa worldwide.

Diseases and pests continue to spread to new areas. For example, the most destructive form of the Ppr disease (*Phytophthora megakarya)*, that causes crop losses of up to 70% in Central Africa, continues to spread westwards in Ghana and has entered Côte d'Ivoire, the world's largest cocoa-producing country. Monilia spread within Central America and into the Peruvian Amazon region in the 1980s and 1990s. Witches' broom spread from the Amazon Region of Brazil to the State of Bahia, being largely responsible for the 50% reduction in the total amount of cocoa produced by the country in the 1990s. In South-East Asia, CPB is spreading within newly established cocoa-growing areas in Indonesia (Sulawesi, Sumatra) and is threatening Papua New Guinea.

Chemical control of diseases or pests is often not possible (VSD, witches' broom, CSSV) or expensive as frequent spraying is required (CPB, Ppr, mirids). The existing genetic variation for resistance in cocoa collections should be exploited for the development of varieties with effective resistance to pests and diseases.

It can be concluded that the extensive way of growing cocoa is directly related to the production structure, dominated by smallholders. With such a structure, it is difficult to introduce technological changes that depend on increased financial inputs. The introduction and use of improved varieties is therefore one of the most cost-effective and environmentally friendly changes in technology that can be proposed to overcome the major cocoa production constraints.

Seventy percent of the cocoa planting materials used worldwide are still made up of unselected varieties. However, based on knowledge of genetic diversity for all important traits, especially for yield and disease resistance, the potential of improved varieties is considered to be large. Important advances have been made in the 1990s in developing more reliable early screening tests for resistance to diseases and pests, especially for the black pod (*Phytophthora* pod rot or Ppr) and witches' broom diseases.

Many cocoa-producing countries have been unable to maintain effective long-term breeding activities because of their limited financial resources, especially during the period of low cocoa prices in the 1990s. In addition, many breeders lack training and operate under isolated conditions; thus international cooperation in cocoa breeding is needed. This was the main reason for the creation, in 1994, of the International Group for Genetic Improvement of Cocoa (INGENIC). INGENIC was closely consulted during the development of the project proposal.

Against the aforementioned background information the project has been justified as follows:

a) Cocoa is, and for a considerable time is expected to continue to be, predominantly an extensively grown crop produced mostly by smallholders;
b) One of the most efficient ways to enhance sustainable cocoa production is through the creation and use of improved varieties. This technology is easily transferred to smallholders compared to other technologies that require higher inputs;
c) Currently grown cocoa varieties show low resistance to major diseases and pests;
d) Genetic progress to overcome major production constraints can be expected through adequate application of proven breeding techniques as well as by additional evaluation of germplasm;
e) Clone selection as well as population breeding strategies appear to be the most appropriate strategies to obtain rapid short-term progress and sustainable long-term genetic progress, respectively;
f) Application of germplasm enhancement procedures (pre-breeding), aiming at improvement of specific traits in breeding populations, is complementary to local breeding efforts in overcoming some of the major cocoa production constraints, such as losses due to diseases. Such a procedure would benefit from additional evaluation studies carried out on germplasm; and
g) International collaboration will be essential to conserve, characterize, select and improve cocoa germplasm in a coordinated manner, and to subsequently distribute it. Because of the existing geographic "division" between areas with cocoa genetic diversity, the cocoa-producing areas and the processing/manufacturing countries, only a global approach will be meaningful.

Project objectives

The ***wider development objective*** was to contribute to the welfare of the large number of smallholders cultivating cocoa through higher and sustainable productivity levels of good quality cocoa at lower production costs, by making optimal use of available cocoa genetic resources.

The ***intermediate project objective*** was to make available improved cocoa planting material with good yield and quality capacity, with resistance to diseases and pests, and well adapted to local conditions.

The ***immediate project objectives*** were:

a) To strengthen national cocoa improvement programmes and increase international collaboration by carrying out cooperative evaluation, selection and breeding activities in ten cocoa-producing countries;
b) To establish cost-effective and efficient conservation, characterization and distribution efforts of available cocoa germplasm; and
c) To strengthen cocoa germplasm utilization and conservation activities through scientific/technical backstopping, information exchange and human capacity building.

Review of project activities planned

The project Components and Outputs related to the ***immediate project objectives*** were identified as follows:

Component 1: International and local clone trials

Multilocational clone trials were to be established in ten cocoa-producing countries, aiming at distributing and evaluating interesting new cocoa clones, selecting superior clonal varieties and assessing the genetic stability of economically important traits. Twenty cocoa clones, supplied by intermediate quarantine centres, were to be compared with 20 local clones in ten different countries for all economically important traits, including disease and pest resistance. For the Ppr and witches' broom diseases, "ring tests" were to be carried out, applying standardized early screening methods, to study stability of these clones to fungal isolates from different geographical origins. In addition, 100-150 interesting trees were to be selected in each country by applying early screening methods. These trees were to be planted in field observation plots.

Component 2: Internationally coordinated hybrid trials

Approximately 40 hybrid progenies were to be produced in each of five countries by making crosses between locally selected superior clones, which are part of the clone trials of Component 1. This would permit the selection of superior hybrid varieties, comparison of the value of the parental clones with their progenies, and selection of individual trees within these hybrids to be used in further breeding. The use of crossing designs would permit genetic analysis of the results to advance knowledge on inheritance of traits.

Component 3: Population breeding

Population breeding programmes were to be initiated or reinforced in four major cocoa-producing countries, aiming at long-term improvement of economically important traits, including disease resistance. The available knowledge about the local germplasm was to be used to identify base populations for initiation of recurrent selection procedures. Exchange of basic breeding material (parental genotypes or seed progenies) was to be promoted between countries which face similar production constraints, thus stimulating regional/international approaches to cocoa breeding.

Component 4: Germplasm enhancement

More heritable economic traits were to be evaluated at the International Cocoa Genebank maintained by CRU in Trinidad (ICG,T), especially resistance to Ppr and witches' broom diseases. Germplasm enhancement consists of identifying more resistant seedlings within crosses between selected resistant clones. This approach was intended to explore the large genetic variation present in this collection to create improved populations. During the project life, a start was to be made on transfering selected clones or populations to user countries.

Component 5: Germplasm conservation, characterization and preliminary evaluation

This component aimed to coordinate and intensify characterization and evaluation of germplasm by identifying genotypes of interest to breeders in international and local collections, with a view to establishing "core collections". Selected material in the ICG,T was to be characterized to evaluate the genetic diversity present in such a core collection (called the "CFC/ICCO/IPGRI Project Collection"). Existing and newly obtained characterization and evaluation results were to be incorporated in the national and international databases. Furthermore, opportunities for collection and conservation of material from interesting new

areas were to be explored. These activities were to be funded by existing or complementary counterpart and co-financing contributions.

Component 6: Distribution and quarantine of interesting genotypes

This component aimed to distribute to participating countries a range of interesting genotypes identified in the project, following the internationally agreed Technical Guidelines for the Safe Movement of Cocoa Germplasm, published by FAO and IBPGR (Frison and Feliu 1989). This included specifically the accessions of the International Clone Trial, of the project core collection to be identified at CRU, and of improved populations. Exchange of cocoa germplasm between the participating institutions was to be stimulated by mutual interests and agreements.

Component 7: Exchange of information and workshops

Exchange of information between project partners was to be achieved through exchange of working documents and through preparation of information sheets, including photographs, on clones to be included in the International Clone Trial and on other widely distributed clones. All the data collected on genotypes were to be entered into the International Cocoa Germplasm Database. Notes on project development and achievements were to be published in newsletters and presented at international conferences, and relevant data introduced into existing databases. A compendium of the results was to be published as a final project publication.

Two project workshops were scheduled: one at the beginning of the project and one at the end of the project. During the first workshop, standardized procedures for evaluation and selection of cocoa genotypes in project trials were to be discussed and adopted and the planned collaborative activities between participants established. During the closing workshop, project results were to be presented and possibilities for project continuation discussed. Regional technical meetings were proposed to be held in the third year of the project.

Component 8: Coordination and scientific/technical backstopping

This component provided the participating institutions with the means and procedures to communicate with each other and cooperate in the various activities. A Project Coordinating Unit was to be established at the headquarters of IPGRI's banana research network (in Montpellier, France), to deal with technical and administrative matters. This Unit was to carry out the liaison between the project partners, needed for efficient implementation of the activities, and to ensure that results are internationally comparable. Working visits of the Project Coordinator to all project sites were to be carried out at regular intervals. Through the exchange of information, through technology transfer, through the visits of the Coordinator to project sites, through the workshops and regional technical meetings, inputs would be made into the strengthening of human capacity in the various disciplinary areas of cocoa breeding and conservation.

Component 9: Management, supervision and evaluation

Day-to-day management of the project was the responsibility of IPGRI, the Project Executing Agency, whereas ICCO was the Supervisory Body. Project evaluation was to be based on 6-month Progress and Financial Reports. A general Mid-term Evaluation would be organized in the third project year.

Project structure

The structure of the CFC/ICCO/IPGRI project (hereinafter referred to as "the project") reflected a multi-stakeholder international collaborative model to achieve a common objective, i.e. making available better cocoa planting materials to farmers. The project brought together important parties in the cocoa production, research and development as well as manufacturing and consumption sectors.

The project was set up as a joint initiative of:

- National research institutes in ten cocoa-producing countries,
- International agricultural research institutes,
- International cocoa genebanks and quarantine centres,
- The International Plant Genetic Resources Institute (IPGRI),
- Chocolate manufacturers' organizations,
- The International Cocoa Organization (ICCO), and
- The Cocoa Producers' Alliance (COPAL).

Collaboration between public organizations and the private sector, i.e. chocolate manufacturers' associations, was fundamental for obtaining the necessary co-financing arrangements. While CFC and counterpart funds were mainly devoted to the improved utilization of cocoa germplasm, the co-financing contributions from the private sector largely supported the conservation, characterization and evaluation efforts.

Research institutes in the ten cocoa-producing countries, representing approximately 80% of world cocoa production, together with the international agricultural research institutes, were the main project implementing agencies (through use of CFC funds, counterpart and co-financing contributions). Within these institutes, the project promoted collaboration between breeders, pathologists, entomologists and agronomists, allowing for integration between these disciplines to achieve common goals.

The collaboration between the project partners promoted international and/or regional approaches to deal with major research and development objectives, such as obtaining varieties with resistance to diseases and pests. The collaboration between the International Cocoa Genebank (ICG,T) in Trinidad, the intermediate quarantine centres in the UK and France, and the research institutes in the ten cocoa-producing countries provided the necessary operational links for more efficient conservation, evaluation, distribution and use of cocoa germplasm.

The co-financing contributions of WCF (formerly ACRI) went mainly to evaluation of witches' broom resistance in Brazil (CEPLAC) and Trinidad (CRU). Co-financing support of BCCCA went to accelerated germplasm conservation, characterization and evaluation activities in Trinidad (CRU), quarantine of cocoa accessions selected for use in the project at the University of Reading, and witches' broom resistance testing (Reading and Trinidad). CIRAD-CP provided important co-financing support by providing scientific staff to reinforce the germplasm characterization and evaluation activities in Trinidad (CRU), the population breeding activities in Côte d'Ivoire (CNRA) and to technical project coordination through an agreement with IPGRI. IPGRI's co-financing support involved project preparation, technical backstopping and project management.

ICCO, representing cocoa-producing and cocoa-consuming countries, provided the political platform needed for evaluation of the project objectives and achievements within its wider commodity development strategy. Furthermore, as the Supervisory Body, ICCO played an important role in the monitoring and mid-term evaluation of the project's progress.

CFC actively participated in the mid-term evaluation of the project through participation in the regional technical meetings and visits of the Project Officer (PO) to several project sites. The PO also participated actively in project steering meetings organized by IPGRI to discuss project implementation aspects together with the Supervisory Body and co-financing institutions.

IPGRI, as Project Executing Agency, prepared all project documents required for efficient implementation of the project. The Project Coordinator visited all project partners on a nearly yearly basis, which was essential for exchange of information and to harmonize the workplans among project partners. Official arrangements with the project partners were made in the form of MoUs (Memoranda of Understanding) and annual LoAs (Letters of Agreement) including, respectively, 5-year and annual workplans and budgets. The Project Coordination Unit created by IPGRI with the support of CIRAD also provided the technical platform for exchange of information and for human capacity building, where required.

A "Technical Working Group" and a "Co-financiers Working Group" were created during the first Project Workshop in 1998 to help steering the project.

Project partners

The participating institutions were:

- **National agricultural research institutions**
  - Cocoa and Coconut Research Institute (CCRI, now CCI), Papua New Guinea
  - Cocoa Research Institute of Nigeria (CRIN), Nigeria
  - Cocoa Research Institute (CRIG), Ghana
  - Comissão Executiva do Plano da Lavoura Cacaueira (CEPLAC), Brazil
  - Centre National de Recherches Agronomiques (CNRA), Côte d'Ivoire
  - Fundación para el Desarrollo de la Ciencia y la Tecnología del Estado de Aragua (FUNDACITE-Aragua) and Fondo Nacional de Investigaciones Agropecuarias (FONAIAP, now Instituto Nacional de Investigaciones Agropecuarias, INIA), Venezuela
  - Institut de Recherche Agricole pour le Développement (IRAD), Cameroon
  - Instituto Nacional de Investigaciones Agropecuarias (INIAP), Ecuador
  - Malaysian Cocoa Board (MCB), Malaysia
  - Ministry of Agriculture, Land and Marine Resources (MALMR), Trinidad and Tobago

- **International agricultural research institutes**
  - Centre de Coopération Internationale en Recherche Agronomique pour le Développement/Département des Cultures Pérennes (CIRAD-CP), France
  - Cocoa Research Unit (CRU) of the University of the West Indies, Trinidad and Tobago
  - University of Reading, United Kingdom

- **Co-financing organizations**
  - American Cocoa Research Institute (ACRI, now World Cocoa Foundation or WCF), USA
  - Biscuit, Cake, Chocolate and Confectionery Alliance (BCCCA), United Kingdom
  - CIRAD, France
  - International Plant Genetic Resources Institute (IPGRI), Italy

- **Supervisory Body**
  - International Cocoa Organization (ICCO), United Kingdom

- **Project Executing Agency**
  - IPGRI, Italy

- **Main financing institution**
  - Common Fund for Commodities (CFC), The Netherlands

Project financing

The accepted project budget, amounting to approximately US$ 10 million, was made up of CFC funding (grant), counterpart funding (in kind) from all participating institutions, and co-financing (in cash and in kind) from ACRI, BCCCA, CIRAD and IPGRI.

At the end of the project, the total project costs, excluding Supervision and Monitoring, have amounted to US$ 11 175 387 instead of US$ 9 907 961 as budgeted in the Project Appraisal Report. The difference of US$ 1 267 426 (12.8% of the budgeted amount) is mainly due to the increases of US$ 481 068 in co-financing contributions and of US$ 813 366 in counterpart contributions. This brings the relative participation of CFC funding, co-financing and counterpart contributions to 25.5%, 21.6% and 52.8% respectively of total project costs, from an original distribution, as reflected in the Appraisal Report, of 28.6%, 19.4% and 51.1%, respectively.

Reference

Frison, E.A. and E. Feliu, editors. 1989. FAO/IBPGR Technical Guidelines for the Safe Movement of Cocoa Germplasm. Food and Agriculture Organization of the United Nations, Rome/International Board of Plant Genetic Resources, Rome.

OUTLINE OF THE CFC/ICCO/IPGRI PROJECT ACHIEVEMENTS

J.A.K. N'Goran[1] and A.B. Eskes[2]

[1] CNRA, 01 BP 1740 Abidjan 01, Côte d'Ivoire

[2] IPGRI/CIRAD, c/o INIBAP, Parc Scientifique Agropolis II, 34397 Montpellier cedex 5, France

Abstract

The CFC/ICCO/IPGRI project on "*Cocoa Germplasm Utilization and Conservation: a Global Approach*" (1998-2003) has significantly increased international collaboration on evaluation, selection and conservation of cocoa germplasm with the aim of producing better varieties. National research institutes in ten cocoa-producing countries, the Cocoa Research Unit (CRU) in Trinidad, the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), France, the University of Reading, UK, and the International Plant Genetic Resources Institute (IPGRI) have participated in the activities. These were characterization, evaluation and selection of promising cocoa genotypes (clones and hybrids), improvement and enhancement of germplasm populations, exchange of information and of selected germplasm accessions between project sites, and transfer and adoption of improved cocoa selection and breeding technologies. Emphasis in the evaluation and selection process has been on disease and pest resistance.

Standardized working procedures for cocoa germplasm evaluation and selection, including disease and pest resistance screening methods, were agreed upon and adopted by the project. Approximately 94 ha of new variety trials (clones and hybrids) have been established at the different project sites. An International Clone Trial was established in nine cocoa-producing countries, permitting the evaluation worldwide of the stability of economically important traits of 20 selected and diverse accessions. Resistance of the "international clones" to the three major species of *Phytophthora* proved to be generally stable in relation to more than 20 fungal isolates from the ten cocoa-producing countries involved. New sources of resistance to black pod disease (*Phytophthora* pod rot or Ppr), witches' broom disease and cocoa mirids have been identified in most cocoa collections. Germplasm enhancement for resistance to *Phytophthora* pod rot has been successfully carried out in Trinidad and Tobago, using the genetic diversity present in the International Cocoa Genebank, Trinidad (ICG,T). The large efforts on germplasm evaluation and information exchange have allowed the identification of an international working collection, called "CFC/ICCO/IPGRI Project Collection", which contains 110 accessions possessing valuable agronomic traits and wide genetic diversity. Distribution of this collection to user countries, through the intermediate quarantine facility at the University of Reading, UK, has been initiated.

The positive effects of the project include increased human capacity building, more effective and coordinated use of limited resources, increased collaboration between cocoa conservation and utilization efforts, and enhanced sustainability of cocoa breeding programmes. The achievements in the current project have permitted the partners to foster further international collaboration and to involve the farmers directly in the process of developing new varieties within the framework of the new CFC/ICCO/IPGRI project on "*Cocoa Productivity and Quality Improvement: a Participatory Approach*", that was initiated in June 2004.

Introduction

The project was set up as a response to the increasing demand arising from cocoa research institutions in developing countries for a sustainable funding of research activities and for better use of cocoa germplasm. One of the main priorities was to evaluate and use germplasm with a higher level of resistance to diseases and pests.

Unfortunately many cocoa-producing countries which could make use of resistant germplasm are unable to maintain effective long-term breeding activities because of their limited financial resources. In addition, many cocoa breeders operate in isolation while they could make use of international collaboration.

The project, whose long-term objectives were to develop and make available improved cocoa varieties, with good yield, quality, and resistance to pests and diseases, appeared as a good start for making better use of the coca germplasm collections and for strengthening collaboration between research institutions.

The project was structured into nine components, of which seven dealt directly with scientific and research activities and the other two with coordination, backstopping and management.

This article provides only a brief summary of the project achievements. These have been described in more detail in the numerous project publications and elsewhere in the present publication.

Project achievements

General outline of results

The activities undertaken were characterization, evaluation and selection of cocoa genotypes (clones and hybrids), improvement and enhancement of germplasm populations, exchange of information and of selected germplasm accessions between project sites, and transfer and adoption of improved cocoa selection and breeding technologies. Emphasis in the evaluation and selection process has been on disease and pest resistance.

The project has achieved all major outputs planned. Besides, more activities were carried out than originally planned. In total, 94 ha of new variety trials (clones and hybrids) have been established at the different project sites, compared to 55 ha originally planned. These contain a total of 2775 clonal and 1647 hybrid varieties (compared to 1300 and 800, respectively, as originally planned).

Standardized working procedures for cocoa germplasm evaluation and selection, including disease and pest resistance screening methods, were agreed upon and adopted by the project during the initial workshop held in January 1998. The publication resulting from this workshop (Eskes *et al*. 2000) was widely distributed (over 400 copies).

The total number of assessments of selection traits carried out on cocoa genotypes in collections and breeding trials in the project have been estimated to be 34 576, distributed as follows: 4877 for vigour; 6026 for yield and related traits; 5000 for self-compatibility; 2385 for pod and bean traits; 150 for fat content; and 16 138 for resistance traits (11 354 to Ppr, 3259 to witches' broom, 630 to mirids, 480 to CSSV, 272 to VSD and 143 to *Ceratocystis*). The evaluation activities resulted in the identification of 2345 promising new selections, each containing one or more favourable traits.

Main results in each project component

Component 1: International Clone Trial (ICT) and Local Clone Trials (LCTs) (11 sites)

Field trials with 20 clones, distributed from intermediate quarantine centres in the UK and in France, were established in nine countries between 1999 and 2004. In Papua New Guinea the trial was planted at two sites to be able to evaluate correctly resistance to VSD. In Cameroon, due to low budding success, the ICT clones could only be established as a Local Clone Observation Plot (LCOP). Evaluation for important traits has been initiated at most sites. In Papua New Guinea and Ecuador significant amounts of data were already collected on vigour, early yield and field resistance.

Resistance of the 20 "international clones" to Ppr has been evaluated by leaf disc inoculation in nine countries, as well as in CIRAD, Montpellier, France, using isolates from the same countries. Good resistance was often observed for PA150, IMC47, PA120, PA107, SCA6, P7, AMAZ15-15, Mocorongo and T85/799. In France, the interactions between clones and fungal isolates, within and between *P. palmivora*, *P. capsici* and *P. megakarya*, appear to be relatively small. However, in Brazil and in Venezuela, most clones, except SCA6, showed low resistance to *P. citrophthora* and *P. palmivora* respectively, indicating some type of interaction. In Papua New Guinea, the results of the detached pod test appeared to give more reliable results than that of the leaf disc test, carried out on grafted nursery plants.

Resistance of clones of the ICT to isolates of the witches' broom fungus was assessed at the University of Reading. Confirmation of isolate-specific resistance was obtained for the SCA6 clone. The level of infection of a series of other clones inoculated with an isolate from Trinidad was quite well correlated with field results from Ecuador.

In nine countries, 20-25 of the most interesting local clones have been planted in Local Clone Trials, with 4-6 replicates. Between 50 and 300 other potentially interesting clones were planted in Local Clone Observation Plots (LCOPs), with one or two replicates only. Use of control clones made comparisons possible between these local clones and the "international clones". Many of the local clones have been screened in the project for resistance to Ppr, witches' broom or mirids. The most promising clones are to be used for further breeding or for testing in farmers' plots.

Component 2: Internationally coordinated Hybrid Trials (HTs) (5 sites)

Between 24 and 92 new hybrid progenies have been produced in Cameroon, Ecuador, Nigeria, Papua New Guinea and Venezuela. The crosses involve mainly locally selected superior clones, which are also part of the LCT. This permits selection of superior hybrid varieties, studies on parent/offspring relationships and selection of individual trees for use in further breeding. Results on early vigour, yield and disease resistance have already been obtained in most countries. Full results can only be expected after five years of field observations.

Component 3: Population breeding (4 sites)

Population breeding programmes have been reinforced or initiated in Brazil, Côte d'Ivoire, Ghana and Malaysia. The aim was to simultaneously improve for economically important traits. The available knowledge about the local germplasm has been used to identify base populations for recurrent selection procedures in each of these countries.

In Brazil, accumulation of genes for resistance to witches' broom and good yield in crosses between genetically diverse populations is the most important objective. The large efforts undertaken include evaluation of individual trees in segregating populations, establishment of observation plots, screening for witches' broom and Ppr resistance, and testing of selected clones in regional on-farm trials.

In Côte d'Ivoire, the first cycle of recurrent selection was concluded and a second cycle initiated (Lachenaud *et al*. 2001). The base populations used are Lower Amazon (plus Trinitario) and Upper Amazon. Selection criteria are yield, Ppr resistance, bean size and yield efficiency. Early screening for Ppr resistance and for attractiveness to mirids was carried out in base populations and for Ppr resistance also in the crosses for the second cycle of recurrent selection (Lachenaud *et al.* 2001; N'Guessan *et al.* 2005). Several selections from French Guiana (GU) appeared promising for Ppr and mirid resistance. Several hundred promising trees, including selections made in farmers' fields, were established in observation plots for further selection.

In Ghana, base populations are IMC, NA, PA and T clones (all Upper Amazon clones). Ppr resistance has been evaluated for several hundred accessions of these populations. More than 500 crosses, within and between groups, were screened for CSSV and Ppr resistance (Adu-Ampomah *et al*. 2005b; Opoku *et al*. 2005). Two hundred progenies were planted in field trials and 100 clones in observation plots.

In Malaysia, base populations (collections) were evaluated for resistance to VSD and Ppr, as well as fat content. Sixty crosses between superior clones were established in variety trials and 97 promising clones (selected from collections and from advanced clone selection trials) in observation plots. Early screening for fat content and for Ppr resistance has been carried out on a number of progenies expected to segregate for these traits.

Component 4: Germplasm enhancement

A germplasm enhancement programme aiming at accumulation of genes for Ppr resistance was initiated using the large diversity in the International Cocoa Genebank, Trinidad (ICG,T). Results were presented in detail by Iwaro *et al.* (2005). Of a total of 960 accessions, 11% were resistant according to the detached pod test, more so in Forastero (14%) than in Refractario (8%) or in Trinitario (6%). Over a 4-year period, 3486 seedlings from 96 bi-parental crosses between 136 clones were screened using leaf disc inoculations. Transgressive segregation for resistance was frequently observed but no immunity. Estimates of heritability suggested that the genetic gain in selecting 10% of the seedlings would result in a population with better average resistance than that of the control (SCA6). In total, 856 selected seedlings have been established in the field during the last three years. A second selection cycle is proposed and the most promising genotypes should then be available to cocoa breeding programmes worldwide.

Component 5: Germplasm conservation, characterization and preliminary evaluation

This component contains characterization and evaluation activities of internationally available germplasm. This activity included mainly the ICG,T managed by CRU in Trinidad, and also studies carried out in several national collections. The studies at CRU involved evaluation of resistance to the Ppr and witches' broom diseases (field studies and studies done by artificial inoculation), studies on genetic markers (isozymes and RAPD) and on pod and bean traits. The number of accessions studied for each of these traits during the 5-year project period varied between 500 and 1000. The results were introduced into the International Cocoa Genetic Database (ICGD) by the University of Reading and were compared to results obtained elsewhere to identify the "CFC/ICCO/IPGRI Project Collection".

The aim of the "CFC/ICCO/IPGRI Project Collection" was to make available to cocoa breeders diverse germplasm that is enriched for important traits, e.g. resistance to diseases, bean weight and pod index (Sounigo *et al.* 2005). The data obtained by CRU over the last 5-10 years were completed with data from other research institutes to identify this collection of approximately 110 genotypes. Molecular marker studies at CRU helped to guarantee the genetic diversity of the collection (Sounigo *et al.* 2001). Most of the selected accessions are part of the ICG,T. By 2004, 40 of the accessions were already available at the quarantine

facility at Reading, and distribution to project partners has been initiated at the occasion of the Closing Project Workshop at Reading in April 2004.

The genetic identity of approximately 30 project clones was verified by using microsatellite markers (Risterucci *et al*. 2001). Over 200 DNA samples from different collections were compared with "control" samples from the intermediate quarantine centres (Reading, UK, and Montpellier, France). On average, 30% of the DNA samples proved to be different among each other or from the control samples. This high level of misidentification demonstrates the need for a continuous effort to verify the identity of cocoa accessions. CRU in Trinidad is already carrying out routine verifications of identity in the ICG,T.

Furthermore, as part of the project, photographs of pods and flush leaves of more than 200 widely distributed clones were made. These have been used by the University of Reading to elaborate a *Guide for visual identification of widely distributed cocoa clones*. This Guide was presented by the University of Reading at the Closing Workshop in March 2004. This guide will be printed and distributed together with the final project report.

Component 6: Distribution and quarantine of interesting genotypes

This component included the maintenance and multiplication of project clones at the intermediate quarantine centres managed by the University of Reading, UK, and by CIRAD, in Montpellier, France. The clones that were part of the ICTs were distributed to all project partners mainly during the first three years of the project. Distribution to other interested institutions was carried out at several occasions.

Exchange of selected germplasm between project sites was carried out based on mutual agreement. Four crosses with resistance to *P. palmivora* were provided by CNRA (Centre National de Recherches Agronomiques, Côte d'Ivoire) to Cameroon and to Nigeria. Leaf inoculation tests showed that these varieties are more resistant to *P. megakarya* than resistant control clones selected in Cameroon (Nyassé *et al*. 2003). The four crosses are currently being validated in farmers' fields in Cameroon and in a variety trial in Nigeria. Four hundred seedlings of a cross between two resistant and productive clones were made available by CCRI (Cocoa and Coconut Research Institute, Papua New Guinea) to CIRAD, France, where they have been screened for resistance to several *Phytophthora* species. The 30 most resistant seedlings were sent to the Reading quarantine centre, and are shortly to become available for distribution to interested user countries.

Component 7: Exchange of information and workshops

Exchange of information between project partners was achieved through exchange of working documents and through preparation of information sheets, including photographs, on clones which are included in the ICT and of other widely distributed clones. All the data collected on genotypes were entered into the International Cocoa Germplasm Database. Notes on project development and achievements were published in newsletters and presented at international conferences, and relevant data introduced into existing databases.

Two project workshops were carried out; one at the beginning of the project and one at the end of the project. The first workshop was held in January 1998 in Montpellier, France, and was attended by 50 participants. Standardized procedures for evaluation and selection of cocoa genotypes in project trials were adopted and the planned collaborative activities between participants were established at this workshop. The proceedings of this workshop were published and widely distributed in 2000 as *Working procedures for cocoa germplasm evaluation and selection* (Eskes *et al*. 2000).

During the First Project Workshop, it was decided to organize mid-term regional technical meetings. These were organized in 2000 by CCRI in PNG (for Asia) and in 2001 by CRU in Trinidad (for the Americas) and CNRA in Côte d'Ivoire (for Africa). These were attended mainly by project scientists but also by representatives of co-financing institutions, CFC,

ICCO and IPGRI. The progress obtained and problems encountered were analyzed and recommendations made on further project implementation.

The Closing Project Workshop, held in Reading in March 2004, was attended by approximately 100 persons representing project partners, co-financing institutions, ICCO, CFC, IPGRI and other interested parties. Project results were summarized and analyzed. The *Final Individual Institute Reports*, compiled by IPGRI in February 2004, were distributed to all partners on the same occasion. A CD-ROM with all presentations given and documents distributed at the workshop was sent in large number of copies to all project partners in June 2004.

Component 8: Coordination and scientific/technical backstopping

The Project Coordinating Unit, established at the headquarters of IPGRI's banana research network (INIBAP, Montpellier, France), dealt with technical and administrative matters. This Unit carried out the liaison between the project partners, needed for efficient implementation of the activities and to ensure that results are internationally comparable. The Project Coordinator visited all project sites at regular intervals. Through the exchange of information, through technology transfer, through the visits of the Coordinator to project sites, as well as through the workshops and regional technical meetings, inputs were made into the strengthening of human capacity in the various disciplinary areas of cocoa breeding and conservation.

Additional activities

Besides the originally planned project activities, the following additional activities were implemented as part of the project as a result of synergies and specific interests of project partners:

- An on-farm trial (IRAD/IITA, Cameroon);
- Budding/grafting trials (CRIN Nigeria, and elsewhere);
- Scion x rootstock trials (CRIG Ghana, INIAP Ecuador);
- Farm selections (CNRA Côte d'Ivoire, CRIN Nigeria);
- SSR marker studies (CNRA Côte d'Ivoire, CIRAD France);
- Visual yield estimation (CNRA Côte d'Ivoire, CIRAD France);
- Pruning trial for *Phytophthora* control (IRAD Cameroon);
- High density trial plots (CRIN Nigeria, INIAP Ecuador);
- Comparison of resistance testing methods for *Phytophthora* pod rot resistance and witches' broom resistance (many partners);
- Comparison of mirid resistance testing methods (CNRA Côte d'Ivoire, IRAD Cameroon, CRIG Ghana);
- An additional replicate of the ICT planted in the Madang area in Papua New Guinea (CCRI, Papua New Guinea); and
- Larger areas and number of genotypes tested in the clone and progeny trials (e.g. by CCRI in Papua New Guinea, CEPLAC in Brazil, CRIG in Ghana, CRU in Trinidad and INIAP in Ecuador).

The additional activities involved also the mid-term meetings in Asia (Papua New Guinea), Africa (Côte d'Ivoire) and America (Trinidad) for exchange of results, reorientation of the project activities and preparation of a follow-up project.

Technical problems and solutions tried

The project has yielded a series of lessons learnt from technical problems. Experiments were carried out trying to overcome these problems. These have often, but not always, produced significant results.

Budding versus top-grafting

In several countries the hypocotyl budding technique recommended at the onset of the project was shown to produce very low success rates (Cameroon, Nigeria and Venezuela). In Nigeria, a series of experiments were carried out to reduce infection of budded plants with opportunistic fungi, and Benomyl at 5% appeared to be quite effective. Despite this result, the use of top-grafting has subsequently been applied in most countries in order to obtain higher average success rate. This method is suited to establish clone observation plots and may be increasingly applied in future by farmers, even in Africa, to multiply preferred clones on their own farm. The top-grafting is however less suited than hypocotyl budding for the establishment of clonal collections, due to the higher chances of sprouting from the rootstock with top-grafted plants.

Clonal multiplication of seedlings

Budding of seedlings, using orthotropic or plagiotropic budwood from selected nursery seedlings or from young trees, has been carried out for "accelerated hybrid clone selection" in Papua New Guinea and in Malaysia. Success of budding has been low in Malaysia, which appears to be due to dormancy of the young orthotropic buds and thus low sprouting of the scion. Results from Papua New Guinea and also from Nigeria suggest that sprouting of the dormant buds can be overcome by eliminating the growing point, thus breaking apical dominance. Top-grafting of seedlings may also be an interesting alternative to seedling budding. Experiments carried out in Malaysia have shown very good results for the bud-break of top-grafted materials in relation to budded seedlings.

Damage due to drought

The dry period of December 2001 to March 2002 was quite severe in some places in Africa. One hybrid trial was lost in Nigeria and another one in the Bechem area in Ghana. These trials could be partially replanted with new seedlings during the reporting period. A small trial carried out at CRIN, using reduction of the leaf surface by 50% before the dry period starts, has not given conclusive results. It appeared that overhead shade was a more important factor to promote seedling survival than reduction of the leaf surface. In Côte d'Ivoire, CNRA put in place a large trial in 2002 to evaluate the effect of leaf surface reduction on survival during the dry period. The results also failed to show any beneficial effect of the leaf surface reduction treatment.

Evaluation of resistance of clones to witches' broom

At CRU, Trinidad, several treatments were undertaken to improve infection rate of inoculations of clones. Manual spraying or drop inoculations of buds appeared to give more regular results than the automized belt spray method. This effect could be due to partial drying-up of inoculum between spraying and incubation of the plants. Results from Reading suggest that latency period may be correlated with infection rate, but also that latency period can be affected significantly by the environment. On the other hand, the weight and length of the brooms did not appear to be affected by the clone, and hence would be less valuable traits to be evaluated.

However, there were other technical problems in the project that could not be solved adequately. The main problem remains the relatively unsatisfactory results from screening for resistance to certain diseases and pests, mainly witches' broom, CSSV and mirids.

Resistance testing methods will therefore receive special attention again in the new CFC/ICCO/IPGRI project on "*Cocoa Productivity and Quality Improvement: a Participatory Approach*".

Indirect benefits of the project

Besides the above direct project achievements, the following indirect benefits of the project should be highlighted:

- Establishment of a worldwide collaborative network on cocoa conservation, evaluation and selection, involving private- as well as public-sector stakeholders;
- Enhanced attention worldwide to the need for development and distribution of better cocoa varieties;
- Increased collaboration between international and national cocoa conservation and breeding programmes;
- Increased multidisciplinary collaboration in cocoa breeding, involving breeders, geneticists, pathologists, entomologists and agronomists;
- Increased human capacity building;
- Transfer of several new technologies and methods in cocoa breeding; and
- More effective and coordinated use of limited resources.

Extent of impact on beneficiaries

The above-mentioned results are very significant and of direct benefit to the community of cocoa breeders, who now have numerous new selections available for further cocoa breeding. The final beneficiaries will be the cocoa producers, although it should be realized that the new selections will not be immediately available, as further confirmation of these selections in the current and in new variety trials needs to be carried out. Many of the selections will however be used in on-farm trials in the new CFC/ICCO/IPGRI project on *"Cocoa Productivity and Quality Improvement: a Participatory Approach"*.

Limitations

Despite the significant benefits obtained, it should be recognized that the project has certainly not been able to cope with all constraints in cocoa breeding. Some of the constraints that could not be addressed at all, or only insufficiently, in the current project are:

- The number of practical cocoa breeders worldwide is very low (about 20). There is an urgent need for more formal training opportunities for young scientists in practical cocoa breeding.
- Results obtained were limited in some places by the limited resources or infrastructure of the collaborating institutes.
- There is an urgent and continuous need to obtain better, high-yielding resistant varieties with good bean quality. Though the current project has provided an important basis for obtaining new varieties in the medium term, the support provided has been far from sufficient in relation to the real needs for large-scale selection of new varieties.
- The defective or non-existent systems of distribution to farmers of improved varieties, where these exist. This problem was not addressed at all by the current project.

Conclusions and recommendations

The project has significantly increased national and international collaborative efforts on evaluation, selection and conservation of cocoa germplasm with the aim of producing better cocoa varieties. The implementation of the CFC/ICCO/IPGRI project activities has been satisfactory at most project sites. The achievements are in general equal or superior to the planned outputs. The quantity of work carried out is impressive and the numerous new cocoa selections made represent an invaluable tool for further and future cocoa breeding. These achievements have also resulted in a better effort to conserve the cocoa genetic resources maintained in the countries. The achievements are in agreement with the high level of resource utilization observed, which is nearly 100% for the CFC funding, 125% for the co-financing and 113% for the counterpart contributions. It should be emphasized that the counterpart contributions have made up the larger part (53%) of the total project costs, which confirms the continuous commitments made by the collaborating institutions throughout the life of the project.

The direct and indirect benefits are expected to enhance sustainability of cocoa breeding programmes. The achievements in the current project will permit the partners to foster further international collaboration and to involve the farmers directly in the process of developing new varieties. Recommendations made during the mid-term project meetings were incorporated in the proposal for a new CFC/ICC/IPGRI project on *"Cocoa Productivity and Quality Improvement: a Participatory Approach"*, which was accepted by CFC for financing in October 2003 and April 2004, and became operational on 1 June 2004. This project will be able to build on the achievements obtained in the *"Germplasm"* project, including the distribution and validation of the most promising selections in farmers' fields.

Despite the progress obtained in the current *"Germplasm"* project, it is important to realize that important constraints continue to prevent good new cocoa varieties from becoming widely available to the farmers. It is hoped that, besides the new CFC/ICCO/IPGRI *"Productivity"* project, complementary funding mechanisms can be found to help to address these constraints.

Acknowledgements

The overview of the project results presented above is based on contributions provided by all technical coordinators of the project in the respective *Final Individual Institute Reports* produced by each collaborating institution and published at the end of the project.

Project publications

The following publications at international level, containing results from project activities, were issued:

Adu-Ampomah, Y., F. Amores, K. Badaru, Y. Efron, J. Engels, A.B. Eskes, E. Frison, V. Gonzalez, P. Hadley, D. Iwaro, K. Lamin, R.A. Lass, K. Maharaj, W. Monteiro, J. N'Goran, S. Nyassé and D. Paulin. 2005a. Results and benefits of the CFC/ICCO/IPGRI project on "Cocoa Germplasm Utilization and Conservation: a Global Approach". Pp. 13-20 *in* Proceedings of the 14th International Cocoa Research Conference, 13-17 October 2003, Accra, Ghana. Vol. 1. Cocoa Producers' Alliance, Lagos, Nigeria.

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N'Guessan, K.F., A.B.Eskes and Ph. Lachenaud. 2005. Study on the resistance of cocoa trees to mirids in Côte d'Ivoire: classification of major groups by their level of resistance/sensitivity. Pp. 559-565 *in* Proceedings of the 14th International Cocoa Research Conference, 13-17 October 2003, Accra, Ghana. Vol. 1. Cocoa Producers' Alliance, Lagos, Nigeria.

Nyassé, S., I.B. Efombagn M., E. Bouambi, M. Ndoumbe-Nkeng and A.B. Eskes. 2003. Early selection for resistance to *Phytophthora megakarya* in local and introduced cocoa varieties in Cameroon. Tropical Science 43:96-102.

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SELECTION AND BREEDING

Rationale of the selection and breeding approaches adopted in the CFC/ICCO/IPGRI project

A.B. Eskes

IPGRI/CIRAD, c/o INIBAP, Parc Scientifique Agropolis II, 34397 Montpellier cedex 5, France

Abstract

Selection of cocoa clones started in Indonesia and Trinidad in the 1930s. Cocoa breeding efforts between the 1960s and 1990s have however mainly relied on the selection of hybrid varieties, generally made up of mixtures of crosses between locally selected and introduced accessions. More recently, clone selection has made a comeback, aiming at quick genetic gains for priority selection traits (productivity, quality, resistance to disease and pests). Main problems encountered in the 1990s were: low adoption rate of improved varieties, limited characterization and evaluation of existing collections, high susceptibility of most planting material to prevailing diseases and pests, low prices of cocoa and, hence, a significant reduction in cocoa breeding efforts worldwide. Studies between the 1970s and the 1990s have indicated substantial variation in germplasm collections, and relative stability and predominantly additive inheritance of selection traits. These findings suggest that good progress can be expected from collaborative efforts in cocoa breeding, from enlarged evaluation of germplasm collections, from selection of clones in superior progenies and from application of breeding approaches allowing for accumulation of favourable genes. These approaches have been at the basis of the main activities proposed in the CFC/ICCO/IPGRI project, i.e. large-scale evaluation of germplasm collections, establishment of clone trials (International Clone Trial, Local Clone Trials and Observation Plots), Hybrid Trials (using best available clones as parents), germplasm enhancement for black pod (*Phytophthora* pod rot or Ppr) resistance and population breeding approaches (involving recurrent selection).

The potential role of cocoa breeding in overcoming production constraints

Most cocoa-growing areas have been developed on forest land, with some forest trees kept to provide shade or using leguminous shade trees. Growing in the full sunlight, a rather recent development, ensures higher yields but calls for higher fertilizer inputs and more regular phytosanitary treatments due to increased insect damage. However, incidence of diseases is generally higher for shaded than unshaded cocoa. About 90% of the world's cocoa is producted by smallholders, especially so in Africa. The use of fertilizers is limited, but that of insecticides and fungicides is more common due to the prevalence of cocoa insects and pests.

Average worldwide cocoa yields are about 400 kg/ha, varying between countries from 150 to about 800 kg/ha (ICCO 1993). However, under experimental conditions and in occasional commercial fields, yields over 2.5 t have been achieved with selected varieties and high levels of inputs. The economic lifespan of a plantation is estimated to be 20 to 30 years, but this can be substantially shortened by destructive diseases and pests. Replacing old cocoa fields with new improved varieties is a continuous challenge for cocoa farmers, hence the importance of an effective and continuing supply of new varieties to the growers.

The extensive way of growing cocoa is directly related to the production structure, predominated by smallholders. Some donors and other international bodies still classify cocoa as a "plantation crop". In practice, cocoa has proved hard to cultivate on a large-holding basis, as illustrated by the uprooting of cocoa on estates in Malaysia during recent years. With the continuing predominance of small cocoa farmers, it is difficult to introduce technological changes that depend on increased financial inputs – especially in periods of

low cocoa prices. The introduction and use of improved varieties continues therefore to be one of the most cost-effective and environmentally friendly technological changes that can be proposed to overcome major cocoa production constraints.

Main production constraints of cocoa are diseases and pests. The most important diseases are Ppr, witches' broom, vascular streak dieback (VSD), moniliasis and the cocoa swollen shoot virus (CSSV). These diseases are expected to continue their spread within the continents affected but might also invade other continents. The most common insect pests are sucking insects, particularly mirids, in Africa, and thrips, in South America. These insects can destroy cocoa plantations exposed to low or no shade conditions, necessitating frequent insecticide applications. In South-East Asia, cocoa pod borer (CPB) causes high crop losses – of up to 90% if no control measures are taken – and is spreading within newly established cocoa-growing areas in Indonesia.

Practically all cultivated varieties, selected or unselected, are very susceptible to diseases and pests of local importance. However, significant genetic variation in resistance level has been found for a number of diseases (Ppr, VSD, witches' broom; and to a lesser extent for CSSV and moniliasis) and pests (mirids, and to a lesser extent pod borer). This shows the large genetic potential to create varieties with increased levels of resistance to diseases and pests. However, the extent of the variation for resistance in collections, breeders' fields and farmers' populations is still not well known. To further explore and speed up selection for genetic resistance, reliable early screening tests are required.

Cocoa varieties and genetic improvement strategies

Less than 40% of world cocoa is estimated to come from varieties selected at research stations. The majority of these are so-called hybrid varieties (mixtures of bi-parental crosses) which are reproduced in seed gardens, generally through hand-pollination. Less than 5% of total cocoa production is provided by selected clonal varieties, propagated as rooted cuttings or as grafts. The rest is still produced by traditional varieties, like Criollo and Trinitario, by slightly improved populations (e.g. open-pollinated Upper or Lower Amazon Forastero populations) or by unselected heterogeneous materials (Eskes and Lanaud 2001).

Cocoa breeding started early this century mainly by clone selection in heterogeneous Trinitario populations, resulting in the identification of some well-yielding clonal varieties still in use today (e.g. in Indonesia, Trinidad and Tobago). Cross progenies among selected Trinitario clones were generally less interesting. After the witches' broom disease appeared in Trinidad, expeditions were organized in the 1930s and early 1940s to search for resistant cocoa trees in Peru and Ecuador. The results of these expeditions were considered disappointing because only very few highly resistant trees could be identified. However, it was soon found that hybrid vigour was expressed in crosses between introduced Forastero clones from the Upper Amazon region and local clones. Such hybrid varieties were tested in many countries and generally provided better establishment capacity, vigour, precocity and yield potential as compared to traditional local varieties. Subsequently, during the last four decades, cocoa breeding has largely depended on selection of crosses between genetically unrelated parental clones. These hybrid varieties have been widely distributed to growers, and today occupy about 35% of total cocoa acreage (Paulin and Eskes 1995; Eskes and Lanaud 2001). The initial success of hybrid selection has meant that for a long time clone selection received little attention in most cocoa-growing countries.

Several shortcomings in conventional hybrid cocoa breeding have been identified during the last two decades (Eskes and Lanaud 2001). The most important problems are heterogeneity of hybrid varieties, composed of mixtures of different crosses, low resistance to diseases and pests and, sometimes, a rapid decline in production capacity with the ageing of plantations. These problems explain why in some countries farmers have abandoned the

use of hybrids and returned to traditional, more uniform varieties. Genetic gains obtained with conventional selection of hybrid varieties are also expected to level off, as they heavily depend on identifying parental clones which combine several desirable traits (high quality, yield and disease resistance) which are often not available or have not been identified in existing germplasm collections (Kennedy *et al*. 1987).

Genetic studies in the cocoa crop have revealed additive inheritance of economically important traits, including disease resistance and yield. Significant correlations between the performance of parental clones and their offspring have been demonstrated (Lockwood and Pang 1994). These findings open new avenues for cocoa breeding, as indicated in the conclusions of the First International Workshop on Cocoa Breeding Strategies organized by INGENIC (the International Group for Genetic Improvement of Cocoa), in particular:

- recognition of the validity of evaluation of clones, to be used directly as commercial varieties or as parents in further breeding;
- possibilities to improve parental genotypes for specific traits through recombination and selection in crosses (hereafter called germplasm enhancement); and
- implementation of population breeding strategies, through recurrent selection procedures, aiming at long-term improvement for all economically important traits.

Therefore, the application of adequate "classical" breeding methods, such as those proposed in the present project, would offer good opportunities for obtaining effective and sustainable progress in this area of applied research.

In some countries, such as Côte d'Ivoire and Malaysia, implementation of recurrent selection or population breeding strategies had begun or was being considered by the mid-1990s (Paulin and Eskes 1995; Eskes and Lanaud 2001). The evaluation, selection and breeding activities proposed in the present project, i.e. multi-locational clonal and progeny trials, germplasm enhancement and initiation of population breeding programmes, are fully in line with these recent developments.

Advances have been made in the 1980s and 1990s in developing more reliable early screening tests for resistance to diseases and pests, especially for Ppr and witches' broom disease. At the same time, more information had become available on the range of the genetic variability available for resistance to diseases in cocoa collections and in breeding fields. The nature of this resistance appears to be quantitative, with additive inheritance predominating. These findings suggest that disease resistance can be accumulated in crosses between less susceptible genotypes. The project proposed that pathologists, entomologists and breeders work together to evaluate all materials used in project trials by applying existing methods, aiming at rapid use of the most resistant materials in further breeding and selection.

Many cocoa-producing countries have been unable to maintain effective long-term breeding activities because of their limited financial resources, especially during the period of low cocoa prices in the 1990s. The continuity of several breeding programmes was endangered and a few were effectively abandoned during the 1990s, as is the case for the cocoa breeding programmes funded by the private sector in Malaysia. In addition, many breeders lacked training and operated under isolated conditions; thus international cooperation in cocoa breeding was really needed. This was the main reason for the creation of INGENIC in 1994. INGENIC has been closely involved in the development of the project proposal.

New technologies and cocoa breeding

In recent years, new technologies have evolved which may make significant contributions to improved understanding of the crop and, eventually, to the creation of improved varieties (Eskes and Lanaud 2001). The advances of DNA marker technology have two important applications. First, it increases our knowledge of the genetic diversity of cocoa germplasm and thus facilitates the application of better-oriented breeding strategies. Second, the recent establishment of a genetic linkage map using these markers makes it possible to apply "marker-assisted selection" once significant correlation between DNA markers and selection traits have been identified (Lanaud *et al.* 1995). This would allow for increased selection efficiency in progenies in which enough polymorphism for DNA markers is present. Cooperation between countries is useful to further develop this technology, making it more cost-effective and more widely applicable. Advances in genetic transformation potentially allow for use of "foreign DNA" in tackling problems such as resistance to CSSV and CPB. However, the relatively limited progress in cocoa micropropagation, regeneration and transformation techniques makes it unlikely that this method will be available to overcome these production constraints within the near future.

Evaluation and use of genetic diversity

Any cocoa breeding programme depends on the availability of useful genetic diversity. Such diversity can either be obtained from nature (usually "wild" genotypes), from farmers' fields (traditional local varieties or diversity present in more recently distributed varieties), or in research institutes, where crosses between different genotypes lead to new genetic combinations. Cocoa breeders have been creating working collections for over 60 years. It is estimated that the collections worldwide contain currently about 7000 original accessions. There is a threat of loss of diversity in germplasm collections, not only due to natural calamities (fire, diseases), but also due to irregular funding.

Cocoa originated in South America and developed its genetic diversity over thousands of years in that continent. Consequently, the existing international germplasm collections have been established in that part of the world (CRU (Cocoa Research Unit) in Trinidad and CATIE (Centro Agronómico Tropical de Investigación y Enseñanza) in Costa Rica). These collections contain approximately 3000 and 800 accessions. In addition, important national collections exist in the countries where cocoa naturally occurs or where it has been cultivated for a long time, including Brazil, Ecuador, Venezuela, Colombia and Mexico. Since the boom of cocoa as a new crop in West Africa and South-East Asia, breeding collections have been built up in these countries, including local selections and introduced germplasm from Latin America (Eskes and Lanaud 2001).

Only a very low percentage of the unique genotypes held in the international and national collections had been evaluated by the beginning of the 1990s. It has been estimated that less than 5% has been used as parents to select new varieties, mainly in breeding programmes aiming at higher yield potential (Paulin and Eskes 1995). The recent emphasis in cocoa breeding is to combine yield with other important traits, such as quality and disease resistance (Eskes and Lanaud 2001). Most of the smaller national collections lack sufficient genetic variability for these traits and so international collaboration is essential to identify and disseminate interesting clones to be used in breeding programmes.

The project proposed to effectively link the characterization and evaluation of internationally available germplasm directly to the needs of breeders through a well-coordinated cooperative effort between researchers, breeders and curators working either in international collections or in the user countries.

Specific objectives of selection and breeding activities

Against the aforementioned background, the breeding and selection activities supported by the CFC/ICCO/IPGRI project included proven classical methods in cocoa breeding and selection, which could be applied in the countries participating in the project. The specific objectives of each of the breeding and selection activities were as follows:

- The ***International and Local Clone Trials (ICT and LCTs, 10 sites)*** aimed at distribution of interesting new cocoa clones, assessment of the genetic stability of economically important traits, and selection of superior clonal varieties for further use in breeding or as candidates for new clonal varieties;
- The ***Local Clone Observation Plots (LCOPs, 10 sites)***, aimed at identification of new promising clones within superior hybrid progenies, within farmers' fields or within local germplasm collections;
- The ***Hybrid Trials (HTs, 5 sites)*** aimed at selection of improved hybrid varieties and at increased knowledge on inheritance of traits (by comparing the performance of parental clones with that of the progenies);
- The strengthening of ***germplasm enhancement*** activities (or pre-breeding), already initiated in the mid-1990s at the Cocoa Research Unit (CRU) in Trinidad, aimed at obtaining populations with increased Ppr and witches' broom resistance, to be distributed to user countries using the large genetic diversity available in the International Cocoa Genebank maintained by CRU; and
- The initiation and/or reinforcement of ***population breeding programmes (4 sites),*** aimed at long-term cumulative improvement of economically important traits, including disease resistance.

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Population breeding activities in Brazil

W.R. Monteiro, U.V. Lopes, J.L. Pires, M.M. Yamada, J.R.B. Marques, E.D.M.N. Luz, S.D.V. Midlej and M.C.A. Paim

CEPEC/CEPLAC, CP 07, 45600-970, Itabuna, Bahia, Brazil

Abstract

At the end of the 1980s in Brazilian farms the productivity was very low, mainly due to the low price of cocoa, the varieties planted and inadequate management of the plantations. This was aggravated by the introduction of witches' broom in 1989, which reduced the country's production of dried cocoa by 75%. Many control measures were attempted to minimize the impact of the disease, but resistance was the only one with some level of success. Aiming to make resistant varieties quickly available to farmers, a strategy based on clones was adopted. Initially, this strategy was supported by the variation present in the many breeding populations as well as in farmer plantations. Soon it was realized that a more formal population breeding strategy was necessary. Starting in 1996, with support of the CFC/ICCO/IPGRI project, 112 progenies were produced involving complex crosses (backcrosses, triple crosses, etc.) and nearly 300 plants selected within these progenies to produce the new breeding cycle and with some of them to be tested as clones. At the same time, and still within the framework of the project, a programme of reciprocal recurrent selection was started, based on a factorial mating design involving 16 parents with maximum genetic diversity according to molecular information, high resistance and yield potential. Considerable progress has been achieved using this strategy, but more involvement of the farmers, widening of the genetic base for resistance and prospecting for new germplasm are additional components of the programme that are currently being implemented.

Introduction

In the second half of the 1980s there was a general problem of low productivity of the majority of the cocoa plantations in Bahia. The productivity of the hybrid varieties until then released to cocoa growers with an expected productivity of 1500 kg of dry beans per hectare was not achieved at the farm level, reaching only 500 kg/ha. The major causes for this low productivity were the quality of the hybrid mixture released as improved planting material, improper management of the cocoa plants, and reduced stand density in the plantations. Before 1984, the hybrid mixture was composed of about 30 hybrid combinations involving self-compatible and self-incompatible clones as parents. There was a large variation for many traits such as yield, pod and seed size, pod and seed number, plant vigour and segregation for alleles of gametic incompatibility. The problems related to cross- and self-pollinations were always present, but until the age of 8 years the direct and indirect effects of incompatibility on yield were not easily noticed. After that age, variation in vigour was increased, because the self-incompatible plants dominated the self-compatible ones in growth, increasing the competition among plants in the plantations. The dominated plants became weaker and rachitic and consequently stopped producing. The plant competition resulted in increased mortality of plants. In parallel, the practices of plant management adopted at that time always favoured vigorous trees and in most of the occasions the weaker plants were eliminated for being unproductive. In the attempt to overcome the problems, the hybrid mixture was modified by the exclusion of all hybrids having self-incompatible parents and a more adequate management was given to the plants.

Unfortunately, in 1989 the situation was aggravated by the introduction and spread of witches' broom disease (*Crinipellis perniciosa*, recently renamed *Moniliophthora perniciosa*) in the main cocoa-growing area of Brazil. The severity of the disease was such that the Brazilian yield dropped by almost 75%. The search for new resistant varieties required the implementation of a rehabilitation programme of cocoa plantings (about 300 000 ha). To respond to the cocoa growers' demand, the Cocoa Research Centre (Centro de Pesquisas do Cacau, CEPEC) of CEPLAC had to re-define completely its breeding strategies in order to develop resistant varieties in a very short time.

Strategies for obtaining new productive and resistant varieties

As most of the measures for witches' broom control did not prove sufficiently effective, there was general consensus that the use of resistant varieties would be imperative for controlling the disease. The clone selection strategy seemed to be more appropriate for developing new varieties in a short time, due to the large number of progenies that were already available at that time in field experiments for the development of new populations (Lopes *et al.* 2003).

Aiming to maximize the chances of gain in the long and short terms, and at the same time maintaining flexibility, three strategies of population breeding were adopted at CEPEC. The first strategy was based on selection of clones in farm-populations, aiming mainly at a short-term gain. The second was a non-structured strategy of crossing promising selections of the first bred populations. Single crosses were made between clones, but without the need for crossing them with many other clones as in factorial or diallel mating designs. The third strategy (the second group of bred populations) adopted was a structured strategy. A set of pre-selected clones based on several traits and genetic diversity was intercrossed according to a factorial mating design.

The farm-populations

Due to the great potential that farm-populations presented for the identification and selection of resistant mother trees, they were chosen by the breeders as the first option, because at that time the disease had not yet reached the experimental stations of CEPEC. These farm-populations were composed of a mixture of hybrids and local Amelonado varieties ('Comum' type) previously released by CEPLAC. In this way, they constituted the first base population for clonal selection. The breeders and technicians initiated the identification and selection of resistant cocoa plants and, later on, they were joined by extension agents of CEPLAC and particularly by the producers. These on-farm selections made with the assistance of CEPLAC breeders were termed VB selections. More than 1300 resistant mother trees were identified and evaluated for at least one year. After that, the most promising trees were selected and cloned (Lopes *et al.* 2003, 2005a, c). A cocoa collection with about 300 on-farm selections was established in CEPEC´s Experimental Station and was partially maintained by the CFC/ICCO/IPGRI project. Most of these selections were distributed to cocoa producers to be tested on a small scale as potential planting materials (CEPLAC-CEPEC 2002; Lopes *et al*. 2003, 2005a). As the genetic basis of these on-farm selections for resistance to witches' broom came mainly from SCA6, SCA12 and IMC67, the use of molecular markers became necessary to measure the relatedness of these genotypes in order to identify genetically distant selections to promote varietal diversification in terms of resistance genes (Yamada and Lopes 1999; Faleiro *et al.* 2004). In addition, these selections have been submitted to field evaluation in CEPEC and in some local farms and screened for resistance to witches' broom and *Phytophthora* spp. by artificial inoculation tests. Figures 1, 2 and 3 show examples of results of these screening tests applied to some selections originally selected for resistance to witches' broom.

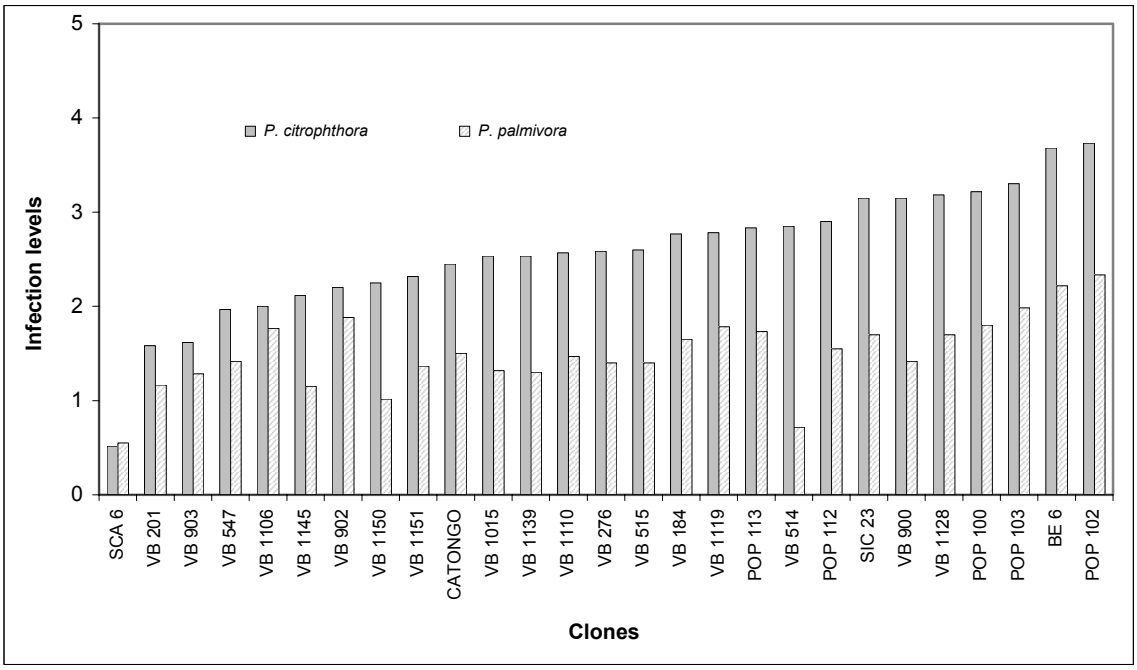

Fig. 1. Average infection levels caused by *Phytophthora citrophthora* and *P. palmivora* on leaf discs of 18 on-farm selections (VB) and 5 other selections from the first bred populations (POP). SCA6 (resistant) , BE6, Catongo and SIC23 (susceptible) were used as controls.

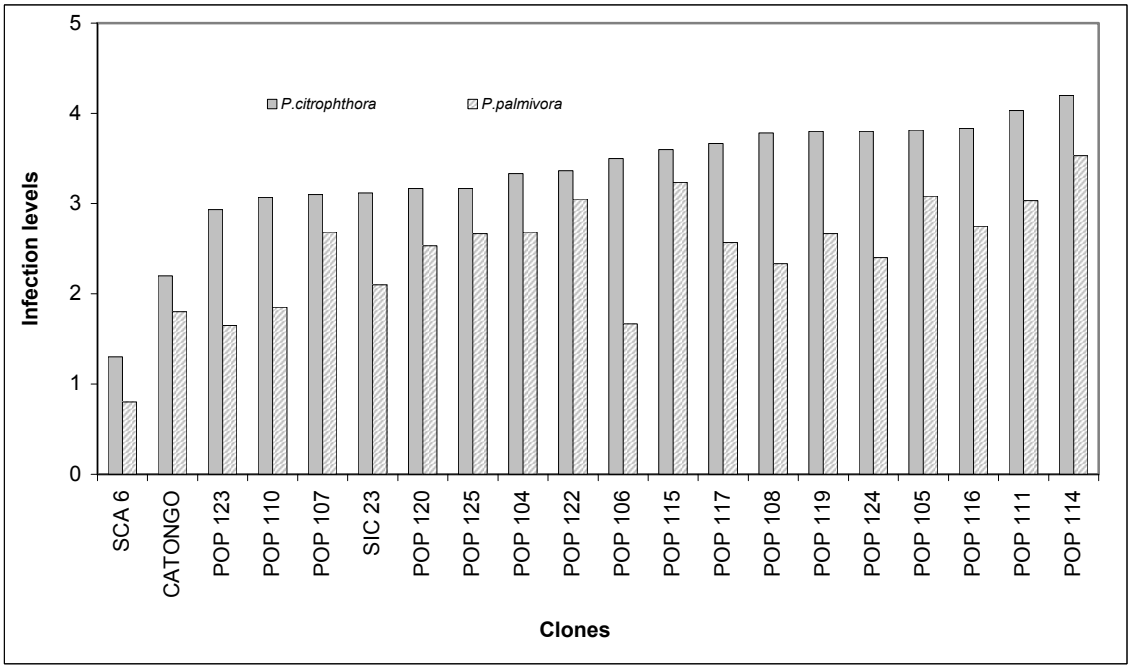

Fig. 2. Average infection levels caused by *P. citrophthora* and *P. palmivora* on leaf discs of 17 different clones selected in the first bred populations (POP). SCA6 (resistant) and Catongo and SIC23 (susceptible) were used as controls.

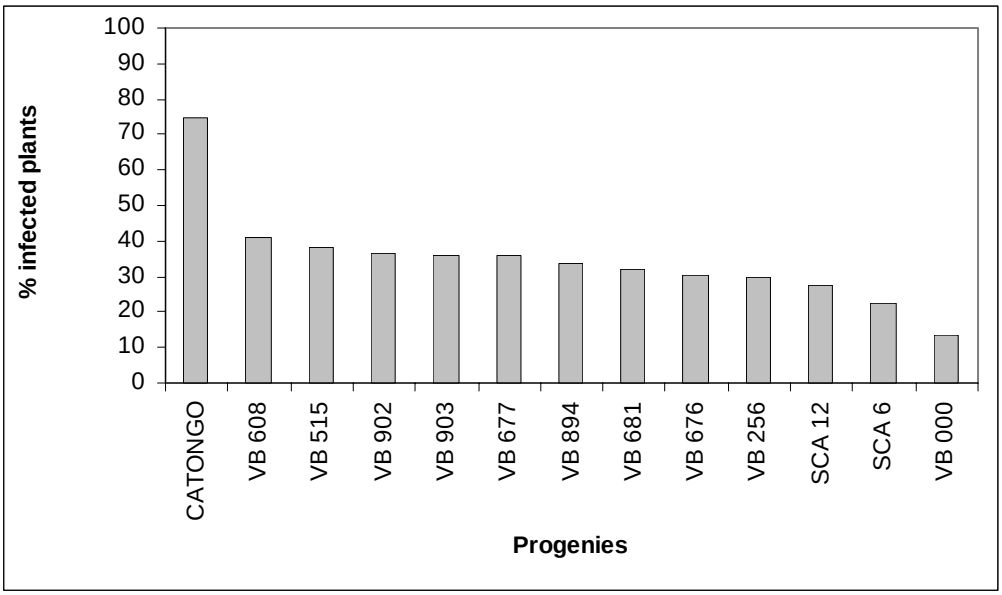

Fig. 3. Percentage of infected plants 60 days after the inoculation with *Crinipellis perniciosa.* Each VB represents an open-pollinated progeny of 112 plants. Catongo, SCA6 and SCA12 were used as controls.

The first bred populations

These populations were bred in 1996 by taking advantage of the infrastructure available at CEPEC at that time, including many hybrid experiments, the germplasm collection and some farm selections. In the hybrid trials, there were many hybrid combinations involving distinct sources of resistance to witches' broom and it was therefore easier to build the base-populations by making two- and three-way crosses, backcrossings and self-pollinations, in order to promote not only improvements for important traits such as yield, seed size and quality, but also to pyramid resistance genes. The pedigree of the parents was always considered when planning these complex crosses. And, as there was no reference in the literature on self-compatible resistant genotypes, the crossings had to be planned aiming also to increase their frequency in the populations. In total, 112 families were produced, involving complex crosses and F_2 generations. Only healthy seedlings from each family were chosen in the nursery to be planted in the field at a spacing of 3.0 x 1.5 m and evaluated individually for resistance to diseases, yield and its components. The high-yielding trees were self-pollinated in order to identify self-compatible genotypes. Individual plant selection for disease resistance and high bean production was successfully carried out in these populations; nearly 300 genotypes could be selected so far. These selections (referred as CP and HB) were cloned and maintained under field evaluation in observation plots. Screening tests for checking the resistance of these genotypes to witches' broom and *Phytophthora* spp. have been applied and the most promising are being tested in multi-location clone trials (Lopes *et al*. 2003, 2005a, b).

As the immediate objective of the clone selection programme was to select a group of genetically diverse clones with higher level of field resistance to these diseases and with the required genetic diversification, some of the self-compatible CP selections were released to cocoa growers for planting (CEPLAC-CEPEC 2002).

Although CEPEC had successfully adopted the above strategies for developing resistant improved clones more rapidly, it was realized that the effect of inbreeding could have played an important role in this breeding process, either by eliminating deleterious genes or by fixing important genes related to resistance to witches' broom and other important traits

in homozygous condition. These populations were investigated in order to acquire more knowledge on how the relatedness of the individuals resulted in an increase in the level of endogamy, and also to measure the effect of inbreeding on many of the traits, especially on resistance to witches' broom (Monteiro *et al.* 2005). The concerns on this matter arose from the fact that many genetically related cocoa clones, regarded as potential sources of resistance to witches' broom disease, were involved. As a result of this study, it was shown that the level of inbreeding was still very low, despite the frequent involvement of some clones, and that it seems to reduce the pod resistance to witches' broom as well as the production of pods and seeds per plant. However, it was observed that the resistance to witches' broom in the canopy and in the floral cushions increased together with the level of endogamy, and that inbreeding had a negative effect on seed weight when more parents with small seed sizes were involved.

Other populations constituted by 112 additional complex crosses were produced in 2003. These were obtained by crosses amongst the individuals selected in each family and also with the new resistant cocoa accessions introduced in the germplasm collection. Only healthy seedlings were taken to the field. These cocoa plants are being individually evaluated in the field in the same fashion as the previous ones and, as expected, the level of resistance of these plants seems to be higher.

The second bred populations

Within a recurrent selection scheme, three subpopulations were bred, involving 16 selected clones from the germplasm collection, by taking into account the field data on yield and resistance to witches' broom and the genetic distance measured at the DNA level by RAPD markers (Pires *et al.* 1999). The mating design presented in Fig. 4 and Table 1 yielded 64 families, forming three subpopulations referred to as A, B and C. According to this mating scheme, subpopulation A is represented by crosses involving eight clones resistant to witches' broom with high genetic diversity and, supposedly, with different genes for resistance (Pires *et al.* 2001; Faleiro *et al.* 2004). Subpopulation B is represented by crosses involving eight clones with high production. Crosses between the resistant clones and the high-yielding clones constitute subpopulation C. This population was taken to the field in 2000, with two replications of 20 individual plants of each family. The scions of each seedling were grafted on basal chupons of adult cocoa trees to speed up the development of the plants and consequently the evaluation for resistance to witches' broom. In the next step within this recurrent selection scheme, plants that will be selected within progenies of populations A and B, according to their principal traits and general combining ability, will be combined in a similar way to form the next generation.

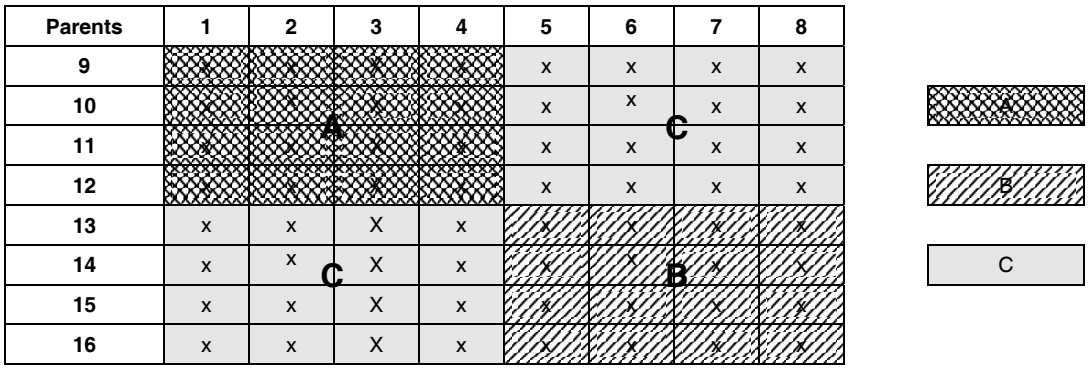

Fig. 4. Factorial mating design for the production of subpopulations A, B and C.

Table 1. Crossing scheme for the production of three base populations for recurrent selection and genetic studies (first value = level of genetic similarity of the two parents based on RAPD markers with 133 polymorphic bands; value within brackets = percentage of seedlings free of witches' broom symptoms)

Parent (*)	**NA33** WBR, BPR, HFC, HFH	**IMC76** WBR, BPR, LSS, LPS	**P4B** WRB, BPR	**CCN10** WBR, LSS, LPS	**CCN51** WBR, HY, LSS, LPS	**CEPEC86** WBR, LSS, LPS	**LSSU54** HY, LSS, LPS	**ICS9** HY, LSS, LPS
SCA6 WBR, HFC	0.59 -	0.57 (93.5)	0.59 -	0.51 (95.0)	0.54 (100.0)	- (100.0)	0.56 (90.7)	0.49 -
Cruzeiro do SUL7 WBR, BPR, HFC, HFH	0.65 (59.3)	0.66 (83.7)	0.66 -	0.56 (90.3)	0.56 (70.0)	- (71.4)	0.61 -	0.58 -
RB39 WBR, HFC	0.61 (95.6)	0.62 (80.6)	0.66 -	0.61 (85.7)	0.60 (80.1)	- (82.7)	0.63 -	0.63 -
CEPEC89 WBR, BPR	0.63 (75.9)	0.72 (78.4)	0.69 -	0.62 (92.5)	0.70 (70.0)	- (82.1)	0.71 (78.8)	0.62 -
OC67 WBR, HY, LPS, LSS	0.54 (75.7)	0.60 (50.0)	0.60 -	0.61 -	0.66 (82.9)	- (63.7)	0.66 (34.9)	0.84 -
BE4 WBR, HY, LSS, LPS	0.70 (60.5)	0.78 (86.1)	0.78 -	0.73 (68.0)	0.77 -	- (53.1)	0.84 (31.4)	0.78 -
EEG29 HY, LSS	0.71 -	0.74 (53.4)	0.78 -	0.77 (90.3)	0.77 -	- (66.7)	0.82 (31.8)	0.78 -
ICS98 HY, LSS, LPS, HFH	0.60 -	0.62 (47.1)	0.64 -	0.62 (76.9)	0.69 (45.5)	- (61.3)	0.71 -	0.84 -

(*) HY = high yield; WBR = witches' broom resistant; BPR = black pod (*Phytophthora* pod rot) resistant; HFC = high fat content; HFH = high fat hardness; LSS = large seed size; LPS = large pod size

CEPEC86 and CEPEC89 are selections of unknown origin

Perspectives

Considerable progress has been made up to now in terms of breeding, but some aspects still need to be worked out more intensively: (i) testing the varieties with farmers' participation, aiming to speed up adoption; (ii) initiating the selection of new clone varieties with a broader base of resistance to diseases such as witches' broom, *Phytophthora* pod rot and *Ceratocystis* wilt in more advanced populations developed in this project; and (iii) prospecting for new planting materials in unexplored areas of the country, mainly in some farmers' populations in the Amazon region.

Acknowledgements

CEPLAC would like to express its gratitude to CFC, ICCO, IPGRI, Co-financers and all partner countries or institutions for providing the necessary financial and technical support for the implementation of the Brazilian activities within this project, which allowed the local breeding programme to obtain significant achievements and to solve many of the major problems with cocoa culture. Special thanks are expressed to Dr Eskes for his competent coordination and friendship, as well as to the Pau Brazil Foundation Team.

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Population breeding approaches applied in cocoa selection in Côte d'Ivoire

J.A.K. N'Goran[1], P. Lachenaud[2], I.B. Kébé[1], K.F. N'Guessan[1], G.M. Tahi[1], D. Pokou[1], O. Sounigo[2], K. N'Goran[1] and A.B. Eskes[2]

[1] CNRA, 01 BP 1740, Abidjan 01, Côte d'Ivoire

[2] CIRAD-CP, TA 80/02, 34398 Montpellier, France

Abstract

Cocoa breeding in Côte d'Ivoire started in the early 1960s as soon as Upper Amazon populations were introduced into Côte d'Ivoire from Ghana. The first crosses with Amelonado and Trinitario genotypes generated a number of selected hybrids that have been distributed to farmers from 1975 onward. In 1990, the need to change the breeding scheme arose in order to better account for resistance traits and to allow for continuous progress in the breeding programme. A reciprocal recurrent selection programme was seen as the best way to accumulate yield resistance traits into progenies. Clones were chosen to be used as parents to create two complementary base populations using information available on the combining ability of the genotypes. Three hundred and ten intra-populations crosses were made and planted in the field from 1991 to 1993. After selecting the best families, individual tree data on yield, yield efficiency and percentage of *Phytophthora* pod rot allowed trees to be selected that were used to create populations of the second cycle of recurrent selection. This second cycle started in 2000. Candidate hybrids from the first cycle, including intra-group and inter-group crosses, are being tested in comparative hybrid trials aiming at possible commercial release.

Introduction

Côte d'Ivoire has become "the country" of cocoa since 1975, when it became the biggest cocoa-producing country in the world. Indeed, 2 million ha of land are planted with cocoa and currently more than 1 million tonnes of dry cocoa are produced each year. Those performance figures hide however a reality, which is the low efficiency of cocoa farms in Côte d'Ivoire. In fact, farmers widely use traditional planting materials that are low-yielding and generally susceptible to biotic constraints. A recent survey conducted at several farms revealed that 25% of them still contained Amelonado cocoa, 62% were planted with mixed cocoa varieties and only 13% were planted with improved hybrids created by the Centre National de Recherches Agronomiques (CNRA). Amelonado cocoa was the earliest introduced cocoa and is considered as traditional cocoa. In addition, most farms are old and so are the farmers.

The traditional cocoa varieties used by farmers in Côte d'Ivoire are low-yielding and susceptible to *Phytophthora* and mirids. Average annual yield nationwide is about 400 kg/ha and the highest average is observed in the south-west region with 660 kg/ha of dry cocoa.

Selection criteria used in the 1960s were yield, earliness and bean weight. Resistance to pests and diseases, based on field observations, were taken into account only as a secondary selection criterion. Those early breeding activities allowed nevertheless the release of 12 hybrids in 1975 (Besse 1977). Those hybrids, although high-yielding, are generally susceptible to *Phytophthora* pod rot and to mirids. Subsequently, new hybrid combinations were tested, resulting in the recommendation of new hybrids that show a slightly higher yield potential and some recommended hybrids also show a higher resistance to *Phytophthora* (Paulin *et al*. 1993; Clément *et al*. 1999).

In 1990, the first population breeding programme was devised and set up to take into account also resistance to diseases and pests as selection criteria from the beginning of the programme. Population breeding was believed to be able to deliver hybrids capable of facing new constraints, e.g. the arrival of the aggressive *P. megakarya* species in the eastern part of Côte d'Ivoire. It was supposed that, by choosing adequate parents, yield could be improved along with disease resistance.

In 1998, when the CFC/ICCO/IPGRI project activities started, the first intra-population crosses were already in the field and were being evaluated. The project provided strong support to the programme under way.

First cycle of recurrent selection

Base populations and layout

The population breeding programme of Côte d'Ivoire was initiated in 1990 and the first trials were planted in the field at Divo in 1991. The programme started as a recurrent selection programme with improvement of two base populations over two cycles (Fig. 1). Based on the progress obtained, including comparison of intra- and inter-population crosses, the scheme could be transformed into a reciprocal recurrent selection programme from the second cycle onwards. The rationale behind the choice of this approach was that it is necessary to recombine favourable alleles in the two base populations through at least two cycles of intra-population crosses, while maintaining a high level of intra-population diversity (Clément *et al*. 1994). Indeed, a close look at early crosses made in the 1960s showed that very few parents (less than 10) were involved in the composition of the hybrids that were released in 1975. The genetic base was therefore narrow and could only allow little progress. In addition, new constraints (such as the arrival of the aggressive *P. megakarya* species in the eastern part of Côte d'Ivoire in the late 1990s) are threatening the cocoa production of Côte d'Ivoire and it was necessary to take them into account from the beginning of the breeding activities.

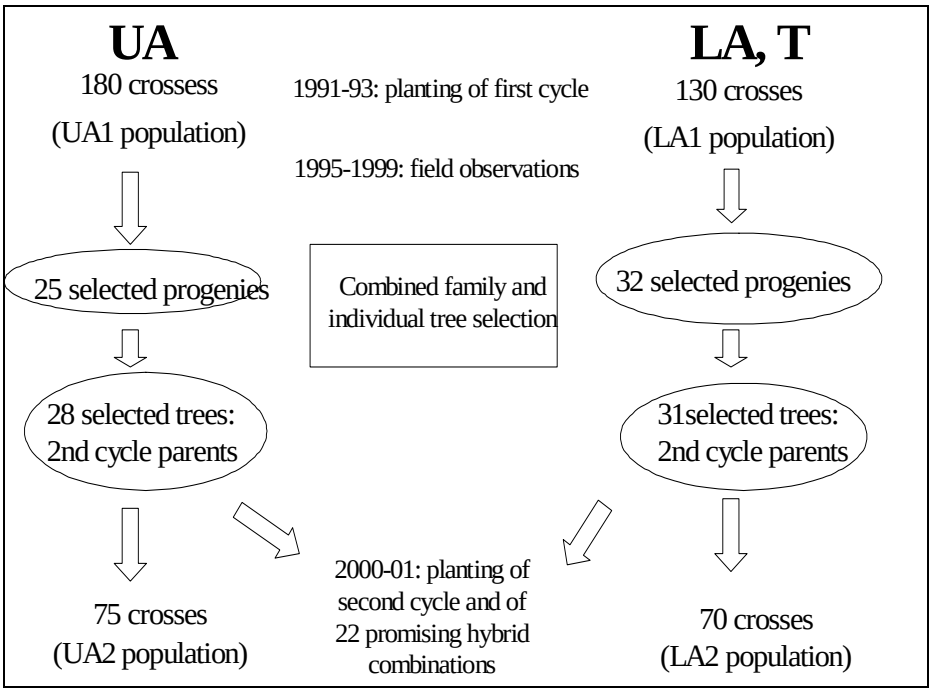

Fig. 1. General scheme of the recurrent selection programme.
UA = Upper Amazon; LA = Lower Amazon; T = Trinitario.

Two base populations, the Upper Amazon (UA0) and the Lower Amazon (LA0), seen as complementary, were selected. The choice of these populations was not arbitrary. In early breeding activities, the best progenies obtained were those resulting from crosses between Upper Amazon clones as female parents and Lower Amazon clones as male parents. Trinitario clones did not appear to be good parents for production. However, in the new population breeding approach, they were used as parents in the Lower Amazon population to improve bean size and adaptation to sunlight.

The Upper Amazon base population (UA0) comprised 20 clones of several UA (Upper Amazon) origins, including Nanays, Parinaris, Scavina, IMC, and 11 diverse clones (mainly near Criollo types). The inclusion of these "Criollo" types was based on the assumption that this group might have originated from some place in the Upper Amazon region. However, genetic diversity data obtained in the 1990s showed that this assumption was a mistake, so the crosses between Criollo and UA genotypes were eliminated from the second selection cycle.

The Lower Amazon base population (LA0) contained 30 clones, mainly LA (African and American Amelonado) but also a number of Trinitario clones.

The choice of clones (Clément *et al*. 1994) was based on their value as clones, their value as parents in inter-group crosses, their geographic origin and also on the genetic diversity as revealed by isozyme markers (Lanaud 1987). It should be noted that, at the time clones were chosen to form the base populations, DNA markers were not available as yet.

Intra-population crosses were made within each base population using hand-pollination after isolation of flowers. Diallel crosses and factorial mating designs were used. Each progeny comprised from 20 to 24 plants that were planted between 1991 and 1993. A total of 310 intra-population crosses, that comprised the "UA1" and "LA1" populations, were evaluated on 5.59 ha (Table 1). Commercially recommended hybrids and promising candidate hybrids were used as controls. Field planting was done using a completely random design of single trees (for the UA0 population) or as units of 4 plants (for the LA0 population). Spacing of plants in the Upper Amazon plot was normal (3 m x 2.5 m), while in the Lower Amazon plot spacing was narrow (3 m x 2 m) (Table 1).

Table 1. First cycle of recurrent selection field trials and layout at Divo, Côte d'Ivoire

Trial and year of planting	Area (ha)	Population	Type of progenies	Field layout
E4/1, 1992	2.64	UA1	Intra-UA and UA x "Criollo" crosses (148 progenies)	Completely random design with 20 trees per progeny
E4/2, 1991	2.25	LA1	Intra-LA and LA x Trinitario crosses (130 progenies)	Randomized plot design with 24 trees per progeny and 4 trees per plot
E4/3, 1993	0.7	UA1	Intra-UA and UA x "Criollo" crosses (32 progenies)	Completely random design with 20 to 24 trees per progeny

Selection criteria

Two levels of selection were applied, firstly selection of superior hybrid families and then of superior plants within superior families. As indicated above, the crosses in the UA1 population including Criollo parents were eliminated from the selection of the UA population.

The criteria used to select superior families from the first cycle of crosses were: survival rate; yield (Lachenaud 1984, 1991); yield/vigour ratio or yield efficiency (Lotodé and Lachenaud 1988); percentage of *Phytophthora*-infected pods; mean pod weight; and level of resistance of the parents to *Phytophthora* estimated by leaf disc tests (Tahi *et al*. 2000).

Superior plants within superior families were selected on the basis of the same criteria, with three additional criteria: level of resistance to pod rot; tolerance to mirids (evaluated according to the method used by Sounigo *et al.* 1999); and breeders' general score of appreciation of the trees (Lachenaud *et al*. 2001).

Results

Genetic analyses of factorial and diallel crosses were conducted after three or four years of observations in the field trials. The results have been presented in more detail by Lachenaud *et al*. (2001) and are summarized as follows:

- Genetic effects (female, male) were generally significant, except for pod losses in the field due to *Phytophthora*;
- Clones known from the literature as good parents for yield were also good parents in the intra-population crosses;
- Several good parents for low percentage of pod rot in the field were also more resistant in leaf disc tests in the UA0 intra-population crosses. However in the LA0 intra-population crosses, little relationship existed between field resistance and the leaf disc test results. This could be due to the fact that the variation for resistance in the LA population was less pronounced than in the UA population.

Table 2 shows that many parental clones interesting for individual selection traits could be identified but that only a few parental clones exhibited at the same time two or more desirable traits, such as yield and resistance to *Phytophthora* disease. Examples of clones combining these two traits were SCA6, PA150 and IMC57. Therefore, the recombination through the intra-population crosses of selected genotypes carried out in the first cycle in the UA1 and LA1 populations is apparently justified to accumulate favourable traits.

Some of the best inter- and intra-group crosses that were evaluated in the field trials (Table 2) showed potential for being selected as candidate hybrids. Seven crosses were selected on the basis of favourable yield, yield efficiency and resistance to *Phytophthora*, in comparison to the control varieties. These crosses are being tested in new field trials as candidates for new hybrid varieties. Four other crosses involving parents with high resistance to *Phytophthora* were also made and are being further tested. The same crosses were distributed to Nigeria and Cameroon where they are being tested for resistance to *P. megakarya*.

Table 2. Best parents selected after evaluation of the first intra-population crosses

Selection trait	Best parental clones
Yield	SCA6, MO81, IMC57, AMAZ15-15, PA150, NA32, IFC303, W41, CC10, ICS84, IFC304, WA40, IFC1, IFC371
Yield/vigour relationship	SCA6, AMAZ15-15, T60/887, PA150, MO81, T79/501, IFC303, CC10, WA40, ICS84, MAT1-6, IFC304
Phytophthora resistance (field infection level)	IMC67, MO98, IMC57, PA4, T60/887, PA150, IMC78, SCA6, IFC6, GS29, VENC4-11, ACU85, IFC14, SNK12, N38, ICS89
Pod weight	IMC78, MO81, AMAZ15-15, IMC6, UPA134, T60/887, SNK12, W41, GS29, CC10, VENC4-11, ICS95

Second cycle of recurrent selection

The best trees from the best intra-population crosses in the UA1 and LA1 populations were selected and used as parents for intra-population crosses to constitute the populations (UA2 and LA2) of the second cycle of recurrent selection (Table 3). These selected parental trees can be considered as improved materials, since they were chosen after the first evaluation phase. Some other UA or LA clones selected in other trials were also included in the second cycle crosses.

In total, 28 parents were chosen from the UA1 population, 6 parents were from old hybrid (UA x UA) trials, 3 from UA selfings and 3 new UA clones from the CNRA collection.

The second cycle intra-Lower Amazon population crosses involved 31 parents from the LA1 crosses, 6 LA parents selected in an observation plot and 3 new local Trinitario parents. Each parent was involved in 4 crosses, composing an incomplete NCII (North Carolina II) crossing design. The field trials planted with these crosses are identified in Table 3.

Table 3. Characteristics of trials in the second-cycle recurrent selection, Côte d'Ivoire

Trial and year of planting	Area (ha)	Site	Population	Number of progenies	Trial layout
E6/1, 2000	1.06	Divo	UA2	75 intra-UA crosses plus 3 control crosses	Single tree randomized design; 15 trees/progeny; 45 trees/control
E6/4, 2000	1.06	Divo	LA2	70 intra-LA crosses plus 3 controls	Single tree randomized design; 16 trees/progeny; 46 or 47 trees/control
E6/5 2002	0.96	Divo	Inter- and intra-population crosses	25 various crosses and 3 controls	Single tree randomized design; 45 trees/progeny; 46 or 48 trees/control
G15, 2001	0.86	Abengourou	Inter- and intra-population crosses	22 various crosses and 3 controls	Single tree randomized design; 45 trees /progeny; 46 or 48 trees/control
E6/6, 2001	0.77	Divo	Inter- and intra-population crosses, selfings	51 various crosses and 2 controls	Single tree randomized design; 15 trees/ progeny; 40 trees/ control

Other activities

The basis of recurrent selection is the existence of genetic variability in each of the participating populations. Therefore, sufficient genetic variability should be available to avoid inbreeding effects due to sampling. In order to maintain and introduce new gene combinations into the reciprocal recurrent selection system, selections were made at farmers' farms and genotypes have been introduced into observation trials to be further used in crosses. Resistant genotypes have been selected and are being further tested. These selected genotypes will be used in the future to introduce resistance traits into new hybrid varieties.

Conclusions

The ongoing population breeding programme is promising. A large number of crosses are being evaluated (Tables 1 and 2). Candidate hybrids are being evaluated in on-station trials and also in on-farm trials initiated recently. Collaboration of farmers in the evaluation of promising hybrids will facilitate diffusion and adoption of new hybrids. The leaf disc test for *Phytophthora* resistance screening, which has been validated during the current CFC/ICCO/IPGRI project, is a valuable tool that has now been integrated into the population breeding programme of the CNRA. It would be useful to have a similar rapid test developed for mirid resistance screening.

Since the beginning of the population breeding programme, new constraints to cocoa cultivation (*P. megakarya* and cocoa swollen shoot virus, CSSV) have been revealed thanks to regular visits to farms. Those threats need to be taken into account by collecting locally adapted germplasm and through regional collaboration.

A possible next step in the programme is the assessment of the genetic gains obtained from the population breeding programme compared with other methods used previously.

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Cocoa population breeding approaches in Ghana

Y. Adu-Ampomah, B. Adomako and I.Y. Opoku
CRIG, PO Box 8, New Tafo-Akim, Ghana

Abstract
Cocoa production in Ghana is faced with many constraints which cause low yields and poor establishment of new plantations. In the past, hybrid varieties (bi-parental crosses) have been developed to deal with these problems. However, there is the need to continually develop more improved varieties for sustainable cocoa production. A project was therefore initiated with the ultimate aim of selecting more useful parents for the development of elite varieties for the Ghanaian farmer. Under this project, clonal and "population breeding" trials were established with the support of the CFC/ICCO/IPGRI project. In both the clonal and population breeding trials the emphasis was on high yield, resistance to *Phytophthora* pod rot, to the cocoa swollen shoot virus and with low levels of attack by insect pests such as capsids. Several new trials are being evaluated concurrently with existing ones, thus increasing the potential of continually developing elite seedling and clonal varieties for farmers.

Introduction
According to farmers, the major constraints of cocoa production in Ghana are low yields and poor establishment of new plantations. The causes of these constraints are attributable to many factors, among which are the inherent low yields of available varieties (approximately 500 kg/ha according to Lockwood (1976) and Adomako and Adu-Ampomah (2000)), high incidence of diseases (*Phytophthora* pod rot (Ppr) and cocoa swollen shoot virus (CSSV)) and pests (especially capsids), and the gradual degradation of the environment, including soils and climatic changes. In the development of improved cocoa planting material these factors are to be considered.

In the past, hybrid varieties have been developed in Ghana to confront these problems and successes have been achieved (Kenten and Lockwood 1977; Lockwood 1981; Adu-Ampomah *et al*. 1999). However, continuous efforts are needed to develop varieties that can outperform those that are currently available in a more sustainable manner. Many accessions of diverse genetic origins have been assembled at the Cocoa Research Institute of Ghana (CRIG) (Lockwood and Gyamfi 1979) but few have so far been effectively utilized for the development of varieties. In this project the approach was threefold:

i. to evaluate parental clones in existing and new trials at both laboratory and field levels for easy establishment ability, early yielding, high yield potential, resistance to prevailing diseases and pests as well as for quality;
ii. to evaluate already available germplasm accessions and newly introduced ones for the same traits; and
iii. to evaluate crosses between and within base populations (genetic groups).

The ultimate objective is to select useful parents for the development of elite varieties for the Ghanaian farmer.

To achieve this objective, clone trials and "population breeding" trials were established with the support of the CFC/ICCO/IPGRI project. The genetic groups used in the population improvement trials are Iquitos Mixed Calabacillo (IMC), Nanay (NA), Parinari (PA) and inter-Amazon selections made by Posnette within the Trinidad introductions (T-clones).

Clone trials

Three clone trials were established, namely the Local Clone Trial (LCT), the Local Clone Observation Plots (LCOPs) and the International Clone Trial (ICT). The main objectives of these trials are to evaluate selected clones as future candidates to be used as clonal planting material for farmers and also in the development of new improved hybrid varieties, with emphasis on high yield, resistance to Ppr and CSSV, and low levels of attack by insect pests such as capsids.

The LCT contains clones that have been selected for good performance in earlier clone trials or which possess interesting traits, such as resistance to Ppr. The expected outcome is to identify candidate clonal varieties for wider field testing, or promising new parents to create superior hybrid varieties.

The LCOPs contains mainly clones from the germplasm collection that have been used as parents in the population breeding progeny trials. These accessions belong to the main genetic groups used in the population breeding approach, and have not been tested before in clone trials. A smaller number of clones in the LCOPs are single-tree selections made within the best hybrid progenies selected in recently finalized hybrid trials. These clones are considered mainly of interest to select new clonal varieties and to compare with their offspring.

The ICT contains mainly clones introduced into Ghana by the CFC/ICCO/IPGRI project. The aim is to evaluate these clones, which were selected for their wide genetic background as well as for useful traits (mainly resistance to Ppr), under Ghanaian growing conditions. Some of the ICT clones have already been used as parents in the population breeding progeny trials.

Progeny trials that are part of the population breeding programme

Four main types of progeny trials were initiated under this programme, namely:

i. The trials of the population breeding scheme (recurrent selection programme);
ii. Comparison of the value of inter-group with intra-group crosses;
iii. A diallel crossing scheme with Upper Amazon clones; and
iv. A trial with "pairwise" crosses among outstanding clones, aiming at recurrent selection of superior clones.

The recurrent selection scheme

The main objective of this scheme is to exploit the genetic variation present in crosses within and between the four genetic groups (Nanay, Parinary, IMC and T-clones). The first cycle of this selection scheme has been initiated and the second cycle is planned (Fig. 1). In Cycle 1, for each of the four base populations approximately 60 selfings and 60 intra-group crosses were made. Furthermore, 180 inter-group crosses were carried out using accessions from the four base populations that were crossed with a tester clone of each of the three other groups. The parental clones and their progenies were evaluated in the laboratory and greenhouse for Ppr and CSSV resistance. Based on these resistance tests and on the field evaluations, Cycle 1 selections within the best selfed families, within the best intra-group crosses and within the best inter-group crosses will be made to create Cycle 2 of the recurrent selection programme.

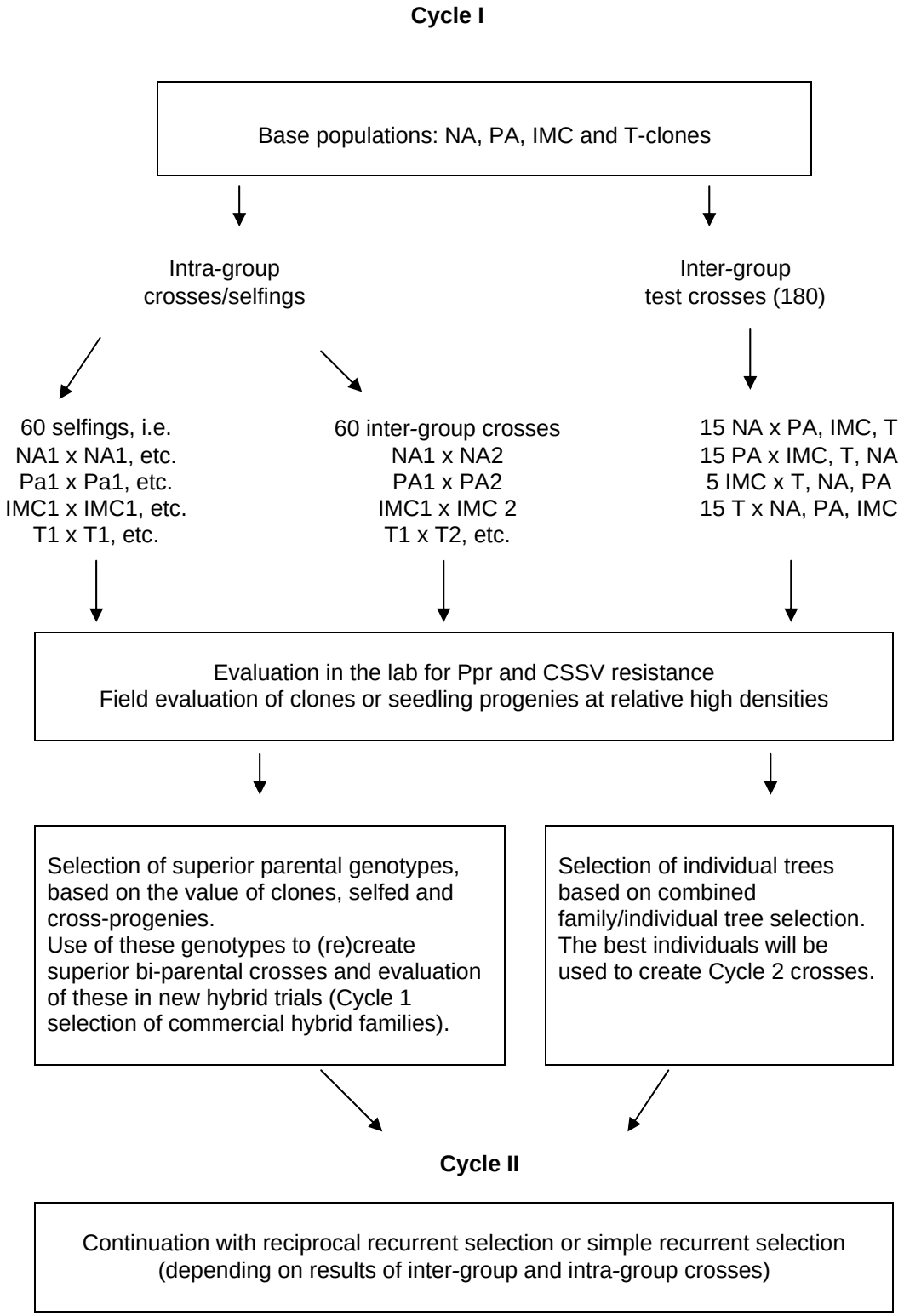

Fig. 1. Reciprocal recurrent selection scheme for cocoa breeding at CRIG.

The best individuals of the Cycle 1 selfings and crosses will be selected on the basis of combined family/individual tree selection, using the laboratory and field data (the latter include vigour, yield, Ppr incidence and CSSV infection, if natural infection is present in the field). The best individuals will be used to create Cycle 2 crosses.

The superior parental clones of Cycle 1 will be used to create new bi-parental crosses, or to recreate the best crosses evaluated in Cycle 1. These will be evaluated in new Cycle 1 hybrid trials aiming at selection and release of superior hybrid varieties for the farmers. Cycle 2 will include a reciprocal recurrent or a simple recurrent selection scheme. This depends on the comparison of the Cycle 1 intra-group and inter-group crosses (see below).

Comparison of the value of inter-group with intra-group crosses in Upper Amazon cocoa

While the ultimate objective of this scheme is to develop new hybrids with improved traits, it is also to ascertain whether the long-term strategy for Upper Amazon improvement should be based on recurrent selection using distinct Upper Amazon populations (NA, PA, IMC and T-clones) or recurrent selection using the Upper Amazon as a group. This will depend on the value of inter-group versus intra-group crosses.

A trial was therefore established based on a factorial crossing design with parental clones of the four base populations: Nanay, Parinary, IMC and Trinidad introductions (Table 1). The crosses were made aiming to have groups with 4 crosses representing inter-group or intra-group combinations. A total number of 40 crosses were made, with the T79/501 x SCA6 cross as control. The parental clones and the progenies were evaluated in the greenhouse for CSSV and Ppr resistance and planted for field evaluation. The best crosses will be used for further evaluation as candidates for new commercial hybrid varieties.

Table 1. Crossing design aiming at the comparison of the value of inter-group with intra-group crosses in Upper Amazon cocoa germplasm

Female	Male									
	PA67	PA151	IMC67	IMC23	NA79	NA151	NA61	T65/238	T63/967	T16/613
PA150	X	X	X	X				X	X	
PA303	X	X	X	X				X	X	
NA33	X		X	X	X	X	X	X	X	
NA34	X	X	X	X	X			X	X	
IMC47				X					X	X
IMC23										X
IMC77			X	X				X	X	
T79/501								X	X	
T85/799								X	X	

Control cross = T79/501 x SCA6

In case of superiority of some of (or all) inter-group crosses, the best base populations will continue to be selected separately and the selected trees within each of the groups will be crossed in Cycle 2 with testers of each of the other groups (reciprocal recurrent selection). In case there is no superiority of inter-group crosses, the genetic groups can be considered as one large Upper Amazon population. The Cycle 2 crosses will then consist of crosses between the best parental clones of Cycle 1 (i.e. the same type of crosses that will be tested as promising new hybrid combinations from Cycle 1 of the population breeding scheme) or between the best individual trees selected in Cycle 1 cross-progenies.

Data (not presented here) on vegetative characteristics and early yield indicate so far that there were no significant differences between the inter-group and intra-group crosses. Therefore, there appeared to be no overall effect of heterosis (hybrid vigour) of the inter-group crosses for vegetative characteristics as well as early yield. If this trend is confirmed, the second cycle should be based on recurrent selection of the Amazon genepool, without distinction of the NA, PA, IMC and T-clones as separate groups.

Diallel crossing scheme with selected Upper Amazon clones

These crosses were made to throw more light on the inheritance of Ppr and CSSV resistance in Amazon crosses involving selected clones (or trees) known to exhibit high levels of resistance to both Ppr and CSSV in field trials. A 6 x 9 factorial crossing scheme was implemented for greenhouse and field evaluations.

Recurrent selection for identification of superior clones

Twenty-four individual trees known to have exhibited high yields in the presence of CSSV and Ppr were selected (from old trials established at Tafo and Apedwa by the Plant Breeding Division of CRIG) and used for the creation of 24 pairwise crosses, using each of the parents in two crosses. The progenies were evaluated both in the greenhouse and in the field for disease resistance, ease of establishment, vigour, yield and field incidence of diseases and pests. The most promising crosses in terms of high yield and low losses from Ppr disease include PA7 x PA150, PA150 x Pound7, Pound7 x Pound10 and Pound10 x Pound15. The result of this trial may permit ortet selection for superior clones, but also the best hybrids will be tested in further trials as candidates for new commercial varieties.

Conclusions

The germplasm collection has substantially been enriched with new introductions from Reading and Montpellier. Several trials from the project are running concurrently with existing ones, thus increasing the potential for developing elite seedling and clonal varieties for farmers. Also the value of already existing varieties has been elucidated in the course of this project, especially in their relative ease of establishment and responses to diseases and pests.

The adoption of the leaf disc test for rapid screening of germplasm for Ppr resistance has had impact on our selection procedure for Ppr resistance. During the course of the project scientists at CRIG have had several positive interactions with cocoa scientists all over the world, especially those in the West African sub-region. The project has also enhanced the multidisciplinary approach to solution of scientific problems at CRIG.

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Germplasm enhancement for resistance to black pod disease

A.D. Iwaro[1], S. Bharath[1], F.L. Bekele[1], D.R. Butler[1] and A.B. Eskes[2]

[1] Cocoa Research Unit (CRU), The University of the West Indies, St. Augustine, Trinidad and Tobago
[2] IPGRI/CIRAD, c/o INIBAP, Parc Scientifique Agropolis II, 34397 Montpellier cedex 5, France

Abstract

A germplasm enhancement programme (GEP) was initiated in 1998 to accumulate resistance genes to black pod disease (*Phytophthora* pod rot or Ppr) by exploiting the rich genetic diversity in the International Cocoa Genebank, Trinidad (ICG,T). During the 5-year period of this programme, 960 genotypes were screened for resistance to Ppr using the detached pod inoculation method. Using data on Ppr and witches' broom (WB) resistance, and pod index, 136 genotypes were selected as base parents in the GEP. Over a 4-year period, 96 progenies were established, and 3486 seedlings were screened for resistance to *Phytophthora palmivora* using a leaf disc method. Among these seedlings, 0.2% (6) were found highly resistant, 11.2% (389) resistant, 52.5% (1829) moderately resistant, 35.3% (1231) susceptible and 0.9% (31) highly susceptible. No immunity was observed. The resistant and moderately resistant seedlings constitute 63.7% of the population evaluated. This may be considered unusual in a crop that is highly vulnerable to *Phytophthora* infection, but it demonstrates the effectiveness of the selection criteria for the base parents, and confirms that resistance to Ppr is heritable. The progeny distributions of most families showed the occurrence of useful transgressive segregates with higher levels of resistance than the parental genotypes. Narrow-sense (h^2_n) and broad-sense (h^2_b) heritabilities were estimated at 0.33 and 0.51, respectively, for a subpopulation of 36 progenies. Using the same population, expected genetic gain was estimated at 0.98. This implies that the selected seedling population would have an average genetic resistance level of 2.42 on a 0-5 resistance rating scale, which is more resistant than SCA6 (2.64), a resistant control clone in the experiment. Among the 3486 seedlings screened, 856, consisting of the resistant and moderately resistant seedlings, have been established in the field. These seedlings are being evaluated for vigour, precocity, Ppr and WB resistance. Further selection will be guided by the expression of these traits in the individual plants. A second cycle of crosses is proposed among the promising resistant genotypes arising from the first cycle. The selection criteria adopted in this programme should facilitate effective selection of promising genotypes/populations with enhanced levels of resistance to Ppr. This material will be made available to national breeding programmes and should allow breeders to combine resistance with good yield potential in new cocoa varieties.

Introduction

Black pod disease or *Phytophthora* pod rot (Ppr), caused by *Phytophthora* species (*P. palmivora*, *P. megakarya*, *P. capsici* and *P. citrophthora*), is one of the most prevalent and destructive diseases of cocoa (*Theobroma cacao*) (Iwaro *et al*. 1998). Global losses from Ppr are enormous and were estimated by Opeke and Gorenz (1974) at about 20-30% of annual cocoa production. However, losses may be as high as 90% at some locations depending on the susceptibility of the cultivated varieties and the prevailing environmental conditions (Adegbola 1981). The disease therefore has a large economic impact on cocoa production and consequently farmers' income. Although chemical control methods have been developed to reduce yield losses from Ppr, they are expensive and often beyond the reach of average cocoa farmers in developing countries (Tan and Tan 1990). The development of high-yielding, resistant material is generally agreed to be a more effective, environment-friendly and

economic control method (Soria 1974; Rocha 1974; Iwaro *et al*. 2000a), but progress in this direction has been very slow due to the narrow genetic base of most cocoa breeding programmes and the low level of resistance in the base parents.

In order to provide cocoa breeders with a wide array of resistance genes, a germplasm enhancement programme (GEP) was initiated in 1998 as part of the CFC/ICCO/IPGRI project on "*Cocoa Germplasm Utilization and Conservation: a Global Approach*". The main objective of the GEP is to develop small populations with high levels of resistance to Ppr. The programme capitalizes on the rich genetic resources at the International Cocoa Genebank, Trinidad (ICG,T) and it is intended to capture as much of its diversity as is consistent with the major objective of enhanced disease resistance. The programme also gives due attention to important agronomic traits.

This paper reviews the progress achieved within the past five years of the GEP at the Cocoa Research Unit (CRU), University of the West Indies, Trinidad.

Methodology

Strategy

The strategy adopted in this programme is outlined below:

- Assessment of pathogen aggressiveness and selection of an isolate for resistance screening;
- Screening of the ICG,T for resistance to Ppr using a detached pod inoculation method;
- Selection of promising genotypes combining resistance to Ppr with other useful agronomic traits;
- Establishment of bi-parental crosses among the selected genotypes;
- Early screening for resistance to Ppr on progeny seedlings;
- Selection of promising resistant seedlings, and their establishment in the field;
- Field observations for vigour, early flowering and resistance to Ppr and WB;
- Selection of the most promising seedlings by detached pod inoculation;
- Commencement of a second cycle; and
- Distribution of the promising genotypes/populations to user countries via intermediate quarantine.

Activities

The following activities were carried out during the 5-year period of the project:

- **Assessment of pathogen aggressiveness and isolate selection**

Five species of *Phytophthora* (*P. palmivora*, *P. megakarya*, *P. capsici*, *P. citrophthora* and *P. megasperma*) have been identified as causal agents of Ppr at different locations (Iwaro *et al*. 1998). *P. palmivora* and *P. capsici* are present in Trinidad and Tobago (Iwaro *et al*. 1998), so we are restricted to these two species for screening cocoa germplasm in Trinidad. Isolates of *P. palmivora* and *P. capsici* were evaluated for their aggressiveness on detached pods, and significant differences were found in their reactions, with *P. palmivora* being the more aggressive (Iwaro *et al*. 1998). Furthermore, there was no interaction between clones and pathogen species, and similar ranking of clones for lesion size for both species allowed resistance to be assessed with one of the two pathogens. Further experiments showed five- to six-fold differences in the aggressiveness of 10 isolates of *P. palmivora* from different locations in Trinidad and Tobago (Surujdeo-Maharaj *et al*. 2001). However, host genotype x isolate interactions were not significant, suggesting that resistance found using any one isolate

would be valid. So far, the ranking of resistance appears to be constant for different isolates and even species of *Phytophthora*. Van der Vossen (1997) reported that the ranking order for resistance to Ppr caused by *P. megakarya* in Cameroon or Togo was very similar to that for Ppr caused by *P. palmivora* in Côte d'Ivoire. These observations suggest that the results of screening for resistance to *Phytophthora* in Trinidad would be relevant to breeding programmes for Ppr resistance at other locations. An aggressive isolate of *P. palmivora* was therefore selected for screening in the GEP. The isolate was activated by passing it through mature cocoa pod after each batch of three inoculation experiments. For long-term preservation of the isolate, cultures were grown on agar slants in McCartney bottles and covered to a depth of 1 cm above the top of the slant with sterile mineral oil (British Pharmacopoeia quality). The McCartney bottles containing the cultures were stored at 25°C.

- **Evaluation of cocoa accessions in the ICG,T for resistance to Ppr**

Nine hundred and sixty genotypes in the ICG,T were evaluated for resistance to Ppr using a spray inoculation method (Iwaro *et al*. 2000b, 2006) on fully-grown, unripe detached pods (4-5 months old). Two to four pods per genotype were tested in each of two experiments to confirm its reaction to *P. palmivora.*

- **Selection of parental genotypes and crossing scheme**

Data from CRU and the International Cocoa Germplasm Database (ICGD) on resistance to Ppr and WB diseases and yield characteristics (bean number, bean weight and pod index) were used to select parental genotypes for the GEP. A total of 136 genotypes were used as base parents in 96 bi-parental crosses (36 Forasteros, 17 Refractarios, 20 Trinitarios and 23 Mixed) within the first four years of the programme (Table 1).

- **Establishment of seedlings and evaluation of progenies for resistance to *P. palmivora***

An average of 30 seedlings per cross were raised in the greenhouse. In addition, 10 replicates of each parental genotype for the year-1 population and two control clones (SCA6 and ICS1) were established in the greenhouse by top-grafting. Six-month-old seedlings from each cross, parental genotypes (for year-1 population) and the control clones (SCA6 and ICS1) were evaluated for resistance to *P. palmivora* by leaf disc inoculation (Nyassé *et al*. 1995), with three repetitions.

Data from the three replicates were subjected to covariance analysis to adjust for differences between test series, using the control clones as covariates. Differences among progenies were assessed by analysis of variance. The narrow-sense heritability (h^2_n) for resistance in the year-1 population of 36 progenies was estimated with a parent-offspring regression. Genetic advance (G_s) was estimated from the standard deviation of the resistance scores (SD) and the broad-sense heritability (h^2_b), with a selection intensity (I) of 10%.

Table 1. Mean values of progenies for leaf resistance to *P. palmivora*

Family	Mean value	Range		S.D.
		Min.	Max.	
Year-1 Population				
IMC47 x ICS41	2.9	1.2	4.9	0.78
B5/3 x ICS41	3.0	1.6	4.4	0.74
NA399 x SCA6	3.0	2.0	4.3	0.59
NA399 x NA672	3.0	2.1	4.0	0.54
ICS53 x NA672	3.1	1.8	4.4	0.64
CL19/10 x ICS40	3.2	2.0	4.6	0.76
ICS53 x SCA6	3.2	1.3	4.6	0.74
NA26 x NA286	3.2	1.7	4.4	0.70
CL19/10 x CL19/49	3.2	1.8	4.5	0.69
ICS75 x ICS40	3.2	2.6	4.2	0.71
M33 x CL19/10	3.3	2.2	4.4	0.58
ICS70 x CL19/10	3.3	1.6	4.4	0.76
NA399 x LP3/5	3.3	1.8	4.3	0.63
B5/7 x SCA6	3.3	1.9	4.6	0.64
NA399 x PA46	3.3	1.9	4.2	0.53
PA125 x NA672	3.3	1.8	4.5	0.69
M33 x IMC2	3.3	1.9	4.4	0.75
ICS75 x NA534	3.3	1.4	4.3	0.84
NA399 x ICS1	3.4	1.9	4.1	0.55
JA5/34 x ICS1	3.4	2.6	4.5	0.56
ICS53 x ICS1	3.4	2.2	4.2	0.61
PA150 x CL19/10	3.4	2.1	5.0	0.72
B5/3 x EET59	3.5	2.5	4.7	0.58
PA125 x SCA6	3.5	2.0	5.0	0.67
PA125 x PA46	3.5	2.2	4.2	0.50
PA125 x ICS29	3.5	2.7	4.9	0.62
NA715 x ICS40	3.6	2.2	4.5	0.66
JA5/34 x NA672	3.6	2.8	4.4	0.49
B5/7 x ICS1	3.6	2.6	4.4	0.51
NA715 x NA534	3.7	2.5	4.8	0.50
ICS70 x ICS72	3.7	2.7	4.7	0.51
B5/7 x NA672	3.7	2.7	4.6	0.63
LX47 x SLC18	3.8	2.1	4.6	0.59
NA26 x SLC18	3.8	2.1	4.5	0.53
ICS10 x ICS29	3.9	2.0	4.8	0.65
ICS46 x SLC18	4.0	3.0	4.6	0.45
Year-2 Population				
IMC105 x PA141	2.9	3.0	4.1	0.37
PA169 x IMC16	3.1	2.3	4.1	0.36
NA104 x PA4	3.1	2.7	4.2	0.37
NA187 x PA218	3.2	2.3	4.2	0.46
AMAZ12 x PA70	3.2	2.3	4.3	0.40
PA124 x IMC103	3.2	2.3	3.9	0.39
TRD28 x ICS69	3.2	2.3	3.8	0.47
PA121 x NA327	3.3	2.2	3.7	0.39
NA184 x Pound7/A	3.4	2.6	4.0	0.32
PA289 x NA141	3.5	2.5	4.3	0.41
PA30 x NA232	3.5	1.8	4.2	0.37
JA3/4 x JA9/16	3.5	2.9	4.4	0.32
Pound26/C x EQX3339/12	3.6	2.4	4.2	0.40
JA5/19 x B7/21	3.6	2.4	4.0	0.31
JA6/4 x CLEM/S-62-2	3.6	2.6	4.3	0.37
CLM96 x B5/7	3.8	2.6	4.1	0.38

Table 1 (cont.). Mean values of progenies for leaf resistance to *P. palmivora*

Family	Mean value	Range		S.D.
		Min.	Max.	
Year-3 Population				
SPA4 x Pound16/B	2.6	1.9	3.5	0.40
NA235 x IMC94	2.6	1.9	3.4	0.32
NA699 x IMC36	2.6	1.4	3.6	0.42
AMAZ6 x IMC27	2.7	2.2	3.4	0.33
NA137 x IMC65	2.7	1.9	2.7	0.40
IMC78 x Pound25/A	2.7	1.7	3.6	0.41
IMC31 x NA702	2.7	1.7	3.7	0.51
SCA6 x SPEC194/109	2.9	1.8	4.0	0.53
CRUZ7/14 x PA34	3.0	2.2	3.8	0.46
NA312 x PA132	3.0	2.0	3.9	0.40
JA4/14 x LP3/5	3.0	1.7	3.8	0.53
ICS45 x TRD32	3.0	2.0	3.9	0.11
ICS45 x EET272	3.1	2.3	4.0	0.49
AM2/19 x LV20	3.1	2.2	4.0	0.47
CL10/5 x AM1/54	3.1	2.3	3.8	0.08
GS12 x TRD32	3.2	1.9	3.8	0.45
COCA3370/5 x NA168	3.2	2.5	3.8	0.40
SPEC18/6 x AMAZ6/3	3.2	2.7	3.7	0.30
ICS45 x TRD13	3.2	2.3	4.1	0.44
LP3/5 x CLM56	3.3	2.2	4.2	0.49
Year-4 Population				
ICS41 x TRD116	2.8	1.3	3.8	0.47
ICS41 x ICS10	2.9	1.2	4.0	0.63
ICS41 x ICS69	3.1	1.6	4.1	0.62
ICS88 x TRD28	3.1	2.0	4.0	0.76
PA70 x NA235	3.2	1.9	4.0	0.52
NA672 x IMC47	3.2	1.5	4.0	0.51
TRD85 x ICS15	3.3	1.7	4.1	0.58
TRD41 x ICS10	3.3	2.6	4.3	0.43
PA150 x IMC76	3.3	2.3	3.9	0.37
NA670 x NA312	3.3	2.2	4.0	0.56
CRU87 x CRU72	3.3	1.8	4.0	0.54
ICS10 x TRD53	3.4	2.0	4.0	0.50
ICS70 x ICS89	3.4	2.3	4.4	0.48
AM2/82 x LP3/5	3.4	2.0	4.1	0.44
TRD32 x TRD116	3.5	2.3	4.1	0.38
TRD88 x GS77	3.5	2.6	4.0	0.40
TRD41 x ICS69	3.5	2.3	4.0	0.51
NA7/10 x IMC20	3.5	1.7	4.1	0.55
B23/2 x JA1/11	3.5	2.3	4.0	0.48
JA3/4 x LP3/5	3.6	2.5	4.1	0.37
B10/25 x MOQ2/18	3.6	2.8	4.3	0.31
CRU19 x CRU100	3.6	2.6	4.1	0.36
TRD32 x GS12	3.7	2.9	4.2	0.31
CL10/5 x MOQ2/18	3.7	2.9	4.4	0.31

Results and discussion

Evaluation of germplasm in the ICG,T for resistance to Ppr

During the first four years of the project, 960 accessions were evaluated for resistance to Ppr (*P. palmivora*) using the detached pod inoculation method (Table 2). One hundred and one (10.5%) were found resistant (disease rating 1-3), 135 (14.1%) moderately resistant (disease rating 4-5) and 724 (75.4%) susceptible (disease rating 6-8) (Table 2). Three hundred and ninety-eight (41.5%) of the total accessions evaluated were Forastero, 291 (30.3%) Refractario, 132 (13.7%) Trinitario and 139 (14.5%) from various origins (Criollo, unknown, various and hybrids). A greater proportion of resistant and moderately resistant accessions was found in the Forastero (14.3% resistant and 16.3% moderately resistant) than in the Refractario (7.6% resistant and 13.7% moderately resistant) and the Trinitario (6.1% resistant and 9.9% moderately resistant). The lower proportion of resistant genotypes observed in the Refractario and the Trinitario groups could be due to different selection criteria imposed on these groups in the past (Iwaro *et al*. 2006). The Refractarios were selected based on their resistance to WB disease, while most of the Trinitarios were selected based on their yield potential. These selection criteria may have limited favourable alleles for Ppr resistance, if these traits are in linkage equilibrium.

Table 2. Distribution of 960 genotypes assessed for resistance to Ppr (*P. palmivora*) at the International Cocoa Genebank, Trinidad

Clone	No. of genotypes assessed	Resistant genotypes (disease rating 1-3)		Moderately resistant genotypes (disease rating 4-5)		Susceptible genotypes (disease rating 6-8)	
		No.	%	No.	%	No.	%
Forastero	398	57	14.3	65	16.3	276	69.4
Refractario	291	22	7.6	40	13.7	229	78.7
Trinitario	132	8	6.1	13	9.9	111	84.0
Others (*)	139	14	10.1	17	12.2	108	77.7
Total	**960**	**101**	**10.5**	**135**	**14.1**	**724**	**75.4**

(*) Amelonado, Comun, Criollo, hybrid, mixed, various, unknown

Detached pod inoculation
Inoculum concentration = 100 000 zoospores/ml

Disease rating

1 = no visible lesion
2 = 1-5 localized lesions
3 = 6-15 localized lesions
4 = >15 localized lesions
5 = 1-5 expanding lesions
6 = 6-15 expanding lesions
7 = >15 expanding lesions
8 = fast expanding coalesced lesions

Marked differences were observed among 11 accession groups, each of which was represented by at least 20 genotypes (Fig. 1). The greatest proportions of moderately resistant and resistant genotypes were recorded in the PA and NA accession groups in the Forasteros. TRD was the most promising group in the Trinitarios, while MOQ had the greatest proportion of resistant and moderately resistant accessions in the Refractarios. Earlier investigations suggested that the PA and NA groups from the Parinari and Nanay regions are important sources of resistance to Ppr (Soria 1974; Iwaro *et al*. 2003). In addition to these two accession groups from Peru, which are both Forasteros, these results showed that the TRD (Trinitario) and MOQ (Refractario) from Trinidad and Ecuador, respectively, are also good sources of resistance to Ppr of cocoa. The results further showed that different accession groups had varying proportions of resistant and moderately resistant genotypes. This is not unexpected in an outbreeding crop such as cocoa with a high level of heterogeneity. This finding reinforces the idea of a pre-breeding programme (germplasm

enhancement) to accumulate resistance genes over several populations as a strategy for improving the genetic base of resistance in national cocoa breeding programmes.

From this result, promising genotypes combining resistance to Ppr with good yield potential were selected for the germplasm enhancement programme and the "CFC/ICCO/IPGRI Project Collection".

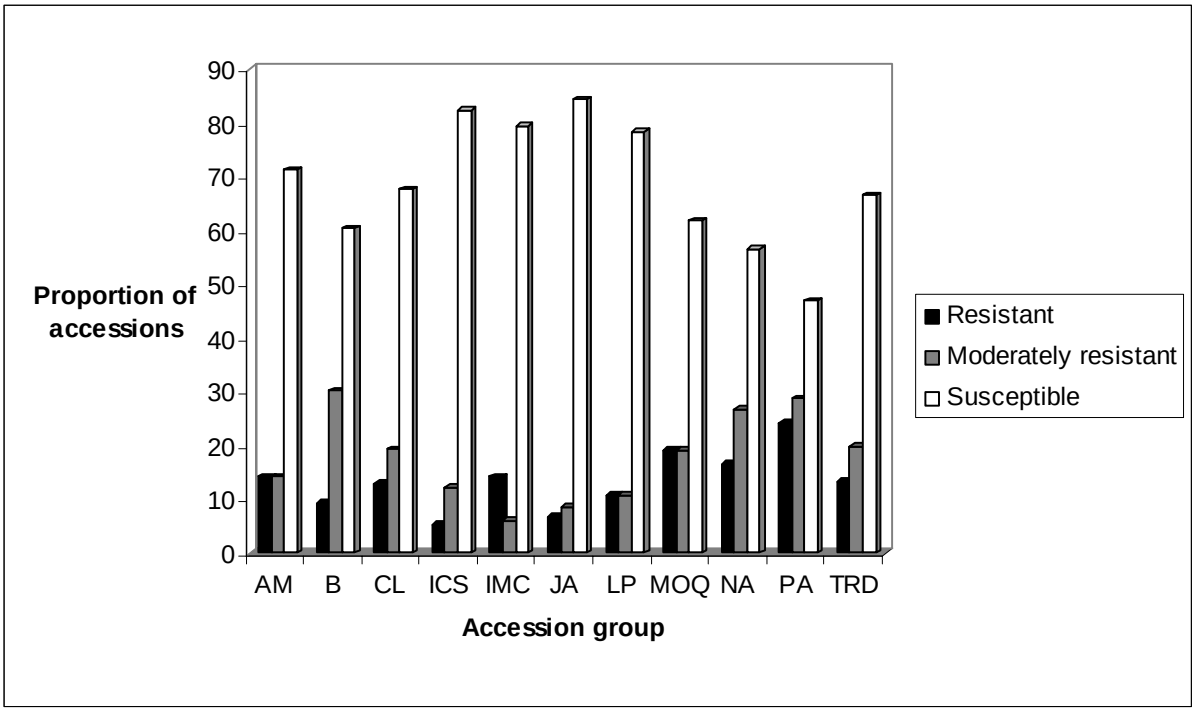

Fig. 1. Distribution of scores for resistance to *P. palmivora* in 11 cocoa accession groups.

Evaluation of progenies for leaf resistance to *P. palmivora*

Ninety-six crosses with 3486 seedlings were evaluated for leaf resistance to *P. palmivora*. The result showed useful transgressive segregates in most families, particularly in crosses with two highly resistant parents, such as IMC47 × ICS41 (Iwaro and Butler 2001). This indicates that resistance genes can be accumulated with careful selection of parents, intercrossing and selection among their progenies. Table 3 shows the distribution of scores for leaf resistance to *P. palmivora* in each of the four populations (years 1-4). Symptoms developed on leaves of all 3486 seedlings indicating that none of the seedlings was immune to Ppr, however, varying levels of resistance were observed among seedlings, in agreement with previous findings (Spence and Bartley 1966; Soria 1974). Six seedlings (0.17%) were found to be highly resistant, 389 (11.16%) resistant, 1829 (52.47%) moderately resistant, 1231 (35.31%) susceptible and 31 (0.89%) highly susceptible (Table 3). Previous inoculation tests on either pods or leaves have normally shown that most cocoa accessions are susceptible to *Phytophthora* (Surujdeo-Maharaj *et al*. 2001; Iwaro *et al*. 2003). However, in this study 52.5% of the seedlings were found to be moderately resistant (Table 3). The resistant and moderately resistant genotypes together form 63.7% of the population, implying an increase in the frequency of resistance genes in the new population. This improvement confirms the effectiveness of the selection criteria and other strategies adopted in the programme. It also shows that resistance to Ppr in cocoa is heritable (Iwaro and Singh 2004).

Table 3. Distribution of scores for leaf resistance to *P. palmivora* among progeny seedlings

Year	Disease rating						Total
	0	1	2	3	4	5	
1	0	3	97	448	432	31	**1011**
2	0	0	28	452	290	0	**770**
3	0	1	201	507	102	0	**811**
4	0	2	63	422	407	0	**894**
Total	0	6	389	1829	1231	31	**3486**
%	0	0.17	11.16	52.47	35.31	0.89	

Leaf disc inoculation
Inoculum concentration – 200 000 zoospores/ml

Disease rating
0 = no symptom
1 = small localized penetration points
2 = small expanding lesions
3 = coalescence of brown spots
4 = expanding lesion
5 = large expanding lesion

Among the four groups of progenies evaluated (Forastero, Trinitario, Refractario or Mixed), higher proportions of resistance and moderately resistant plants were observed among the Forastero progenies as compared to those from Trinitario, Refractario or Mixed parents (Fig. 2). A similar trend was observed among the accessions evaluated for resistance to Ppr in the ICG,T (Table 2). This further shows that resistance is heritable and improved levels of resistance could be achieved in breeding with careful selection of promising resistant parents.

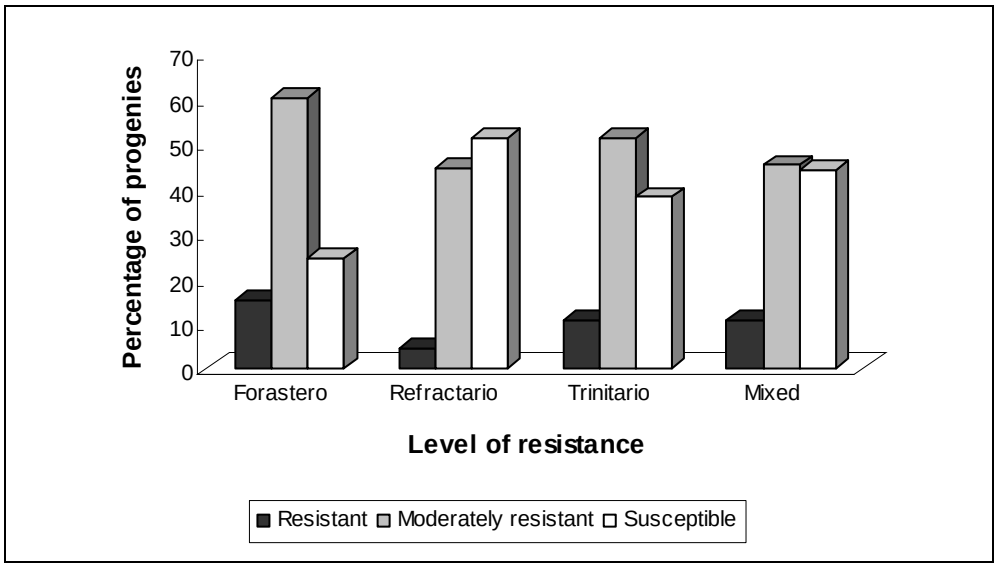

Fig. 2. Distribution of scores for resistance to *P. palmivora* among four groups of progenies.

Narrow-sense (h^2_n) and broad-sense (h^2_b) heritabilities were estimated at 0.33 and 0.51 respectively for the year-1 population of 36 progenies. Genetic gain (G_s) was estimated at 0.98 based on h^2_b (0.51), SD of scores (1.092) and a selection intensity of 10% (1.755). With a genetic gain of 0.98 points on the 0-5 resistance rating scale, the selected seedling population would have an average resistance level of 2.42 (mean of all progenies, 3.4-0.98 expected genetic gain). This is higher than the resistance level of SCA6 (2.64), a resistant control clone.

Establishment of field trial

A total of 856 resistant and moderately resistant seedlings have been established on 1.5 ha of land at the University Cocoa Research Station (Field 7). A second replicate obtained by top-grafting was planted in Centeno (Field 14) under existing cocoa trees to provide high disease pressure for Ppr. Field 7, on the other hand, is a newly established field.

Field observations

Among the year-1 population (246 plants) established in 2000, 129 plants (52%) had flowers, while 78 (32%) were bearing pods during a survey conducted in 2003. In the year-2 population, planted in 2001, 71 plants (27%) were flowering and 42 of these (16%) had pods. Very few plants had flowers and pods in the year-3 and year-4 populations established in 2002. To increase pod production for the evaluation of Ppr, it may be necessary to carry out hand-pollinations. This would facilitate further evaluation of the progenies, parental genotypes and controls, which depends on the availability of pods. About 16% (39 plants) of the year-1 population and 10% (26 plants) of the year-2 population had WB. There was no evidence of WB attack in the newly established populations (year-3 and year-4 populations). Information on WB incidence provides an opportunity for negative selection against WB disease. Further field observations should provide useful information for the selection of promising genotypes with resistance to Ppr and WB.

Future direction

Confirmation of resistance to Ppr in the progeny populations in the field will be obtained using the detached pod inoculation method. A second cycle of the GEP will be carried out as part of the new CFC/ICCO/IPGRI cocoa project, "*Cocoa Productivity and Quality Improvement: a Participatory Approach*". The following strategy would be adopted:

- Selection of 110 resistant genotypes from the first cycle (Forastero: 30, Refractario: 30, Trinitario: 30, Mixed: 20);
- Intercrossing of the selected genotypes;
- Establishment of 55 progenies (2750 seedlings);
- Evaluation of progenies for leaf resistance;
- Establishment of 20% (550 seedlings) in the field;
- Field observations for vigour, early flowering and resistance to Ppr and WB;
- Selection of 110 resistant genotypes for distribution (Forastero: 30, Refractario: 30, Trinitario: 30, Mixed: 20).

The various selection criteria adopted in this programme should facilitate not only effective selection of promising genotypes/populations with enhanced levels of resistance to Ppr, but also selections with good yield potential and field resistance to WB. These will be distributed to national cocoa breeding programmes and should allow breeders to combine disease resistance with good yield potential in new cocoa varieties. This should help to stabilize yield and reduce the need for chemical control of Ppr in many cocoa-producing countries.

Transfer of genotypes/populations to user countries

Genotypes/populations with promising levels of resistance to *Phytophthora* and showing good yield potential will be transferred to user countries following accepted phytosanitary and quarantine measures. The material could be transferred as budwood or seed (crosses between selected parents). The possibility of using disease-free pollen is being investigated. If successful, this would facilitate the transfer of cocoa germplasm between countries interested in the enhanced material.

Acknowledgements

We wish to thank the Common Fund for Commodities (CFC), the Biscuit, Cake, Chocolate and Confectionary Association (BCCCA, UK), and the Government of the Republic of Trinidad and Tobago for financial support. Our sincere gratitude goes to Prof. J. Spence, Mr R.A. Lass and Dr T.N. Sreenivasan for their interest in this work. We are also indebted to Ms V. Singh for assisting with field activities and establishing the GEP database.

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Characterization of germplasm in the International Cocoa Genebank, Trinidad

F.L. Bekele and G.G. Bidaisee

Cocoa Research Unit (CRU), The University of the West Indies, St. Augustine, Trinidad and Tobago

Abstract

Morphological characterization of germplasm from the International Cocoa Genebank, Trinidad (ICG,T) has been in progress for over a decade. By March 2003, fruit data had been collated for 966 accessions. The process of data accumulation has been tedious and time-consuming, and efforts to accelerate it have resulted in a reduction in the sample sizes used for fruit characterization, as reported here. During the period of this CFC/IPGRI/ICCO project, data were presented on bean number, cotyledon weight and pod index for a total of 966 accessions from a diverse array of germplasm from the ICG,T encompassing 69 Accession Groups. Ninety-one of these accessions were found to combine pod index values of ≤20 with a cotyledon weight of ≥0.92 g. The performance of the clones studied has also been examined according to Accession Group, genetic origin and type of germplasm (wild versus cultivated). The Accession Groups JA, CRU, ICS and IMC had considerably more accessions with favourable pod index and cotyledon weight than the other groups studied. Many of the Refractarios differed from the Forasteros in terms of bean number. An apparent improvement in the yield potential of cultivated germplasm has been noted, which appears to be accompanied by an adaptive feature of the breeding system particularly in the Refractarios.

Introduction

Morphological characterization of germplasm in the International Cocoa Genebank, Trinidad (ICG,T) commenced in 1990 at the Cocoa Research Unit (CRU), Trinidad. The main objectives of this project are to describe the 2300 accessions in the ICG,T, compile, analyze and interpret the generated data, and utilize and disseminate the resulting information as described by Bekele (1999). Sixty-nine descriptors from the IBPGR descriptor list for cocoa (1981) were used during the first five years of the project. Based on research at CRU, and after consultation with various experts in the area of cocoa taxonomy and characterization, a concise list of descriptors was developed (Bekele and Bekele 1995, 1996a; Bekele and Butler 2000; Bekele *et al*. 1994a). It was adopted at CRU in May 1995. Related activities include the determination of correlations among the descriptors (Bekele and Richardson-Drakes 1992), assessment of phenotypic diversity within the ICG,T (Bekele and Bekele 1996a; Bekele *et al*. 1994b, 2002a, 2004, 2006), elucidation of a possible association between phenotypic diversity and geographic origin (Bekele and Bekele 1996a; Bekele *et al*. 2006), evaluation of the impact of environment on the expression of some fruit and seed characters (Bekele and Bekele 1996b), examination of the variation in pod wall hardness among accessions (Bekele *et al*. 1997a), evaluation of characters of economic interest such as pod index, bean number and cotyledon weight (Bekele *et al*. 1995, 1997b, 1998, 1999, 2000, 2001, 2002b, 2003), and comparisons of classifications obtained using morphological and biochemical/molecular data (Sounigo *et al*. 1998). Our current activities include routine characterization using 26 descriptors (Bekele *et al*. 2006), photography of mature fruits (ripe and unripe), preparation of manuscripts on Accession Groups of special interest for publication in the International Cocoa Germplasm Database (ICGD) and elsewhere, and collaborative evaluation studies including those to identify accessions with favourable yield potential and resistance to black

pod disease (*Phytophthora* pod rot or Ppr) (Iwaro *et al*. 2001, 2002, 2005), and aimed at verification of clone identities (Christopher *et al*. 1999; Boccara *et al*. 2002).

During the period of the CFC/IPGRI/ICCO project on *Cocoa Germplasm Utilization and Conservation: a Global Approach*, data were presented on bean number, cotyledon weight and pod index (PI) for a total of 966 accessions from a diverse array of germplasm from the ICG,T encompassing 69 Accession Groups. Ninety-one of these accessions were found to combine pod index values of ≤20 with a cotyledon weight of ≥0.92 g.

Methods

Fruit data collation was systematic, involving accessions for which plant material was available during surveys of the collection, and designed to include as many of the Accession Groups represented at the ICG,T as possible. Sample collection spanned the period January 1992-March 2003. The methods used during the project were as described by Bekele and Butler (2000) in the *Working Procedures* (pp. 41-48); Bekele and Bekele (1996a); Iwaro *et al*. (2003); and hereunder. There was one modification, which was implemented during the course of the project, *viz*. a reduction in sample size.

Reduction of sample size used during the course of the project

In an effort to reduce the time required to characterize pods (fruits), a study was undertaken to determine whether the sample size used could be reduced without loss of statistical precision (Bekele *et al*. 2000). The mean PI values generated for 400 accessions using a sample size of 20 were compared with those resulting from the use of sample sizes of 10 and 5, respectively. There were statistically different values for the means generated using a sample size of 20 compared to one of 5 based on a paired t-test ($t=3.88$, $P<0.0001$). However, there was no statistically significant difference in the means generated using sample sizes of 10 and 20 ($t=-2.88$, $P>0.05$). Accessions with PI less than 20 (pod sample size = 20) are listed in Table 1. The corresponding PI values with a sample size of 10 are also presented. Based on these results, a sample size of 10 was adopted for fruit characterization in March 2000.

Recording fruit and seed descriptors

Ten healthy, well developed, ripe pods were harvested per accession. If the complete sample was not obtained at one picking, every effort was made to obtain the full complement within one harvest period.

The pods were assessed in terms of shape, surface texture (rugosity), husk hardness, colour and disposition of ridges, basal constriction and apex form. The length and width were measured. The pods were then opened and the wet seed mass was removed and separated from the central placenta. The number of seeds per pod (excluding flats) and the number of flats were counted. Pods with an inordinately large number of flats were discarded. The mucilage surrounding the seeds of each pod was removed by washing with a high pressure hydro-jet after the seeds had been pre-treated with calcium oxide.

The weight of the cleaned, air-dried seeds of each pod was recorded using a Sartorius balance (2000 g capacity). The seed samples were then dried in an oven at 105°C for 24 h. The testas were removed from the dried seeds and the individual weights of 20 randomly selected cotyledons (two from each of 10 pods) were determined using an Ohaus 40 000D balance. The pod index was then calculated by dividing 1000 by the product of average bean number and average dried cotyledon weight.

Table 1. A comparison of the pod index values (PI) obtained with sample sizes of 20 and 10, respectively for accessions with PI less than 20 for the larger sample size

Accession	Pod index n = 20	Pod index n = 10
UF11	14.87	13.94
ICS68	15.55	15.87
UF12	15.57	14.87
JA5/36	15.75	15.53
JA5/31	16.46	17.23
ICS60	16.54	15.63
ICS5	16.98	16.98
IMC97	17.09	16.00
LP3/40	17.31	17.66
Silecia8 (EET395)	17.38	16.23
EET59	17.61	23.45
Pound18	17.68	17.77
ICS94	18.07	19.67
AM2/91	18.19	17.24
JA5/5	18.27	19.70
IMC3	18.74	18.67
Matina1/7	18.77	19.03
EET397	18.94	19.05
ICS43	18.99	16.05
GS29	19.03	17.55
IMC65	19.05	18.87
ICS75	19.07	18.66
IMC10	19.07	16.90
IMC68	19.25	20.01
CL27/50	19.57	18.66
ICS111	19.58	19.03
UF29	19.58	19.32
ICS1	19.69	19.88
SCA9	19.70	20.32
SJ2/22	19.94	19.43

Descriptive statistics

Variable	N	Mean	Standard deviation	Standard error
Pod index (n = 20)	400	27.535	6.255	0.313
Pod index (n = 10)	400	27.865	6.832	0.342

Outputs achieved over the duration of the project

Main results

- Bean numbers ranged from 17 for B5/11 to 59 for IMC39, with a mean value of 39.6±0.19. Four hundred and seventy-six accessions had bean numbers equal to or greater than 40. One hundred and seventy-eight accessions had ≥45 beans and fifty accessions had ≥50 beans.
- Dried bean weights ranged from 0.44 g for B10/28 and PA46 to 1.84 g for UF11, with a mean value of 0.97±0.006. One hundred and twenty-eight accessions had bean weights equal to or greater than 1.2 g. Sixty-seven accessions had bean weights of 1.29 g or greater and ten had bean weights ≥1.5 g.
- The pod index values ranged from 13.9 for UF11 to 92.8 for B9/10/35, with a mean value of 27.8±0.23. Ninety-one accessions had pod indices ≤20 (Table 2).

- Twenty-five (36%) of the 69 Accession Groups characterized had one or more accessions with favourable pod index and bean weight values. Of these, JA, CRU, ICS and IMC had considerably more accessions satisfying these criteria (Fig. 1). These Accession Groups are apparently good sources of genes for large bean weight and low pod index.
- There were significant differences ($P \leq 0.0001$) among the groups of cocoa studied in terms of the traits under investigation. A study of a subset of 613 accessions revealed that many of the Refractarios differed from the Forasteros in terms of bean number. In addition, the Refractario population JA differed from LP and B, also Refractarios, in terms of this trait. The Forastero population IMC also differed from ICS and GS (Trinitarios) in terms of bean number. The IMC population was distinctive for large bean numbers. ICS and B differed significantly in all of the traits studied. ICS had the heaviest beans (α=1.2 g) and Pound the lightest (α=0.87 g).

Table 2. Accessions with favourable pod and bean characteristics

Accession	Bean number	Cotyledon weight	Pod index
UF11	39	1.84	13.9
UF12	38	1.77	14.9
CRU147	46	1.43	15.2
JA5/36	46	1.40	15.6
CRU34	47	1.37	15.5
ICS60	39	1.64	15.6
CRU138	47	1.35	15.8
JA5/7	45	1.41	15.8
ICS68	50	1.26	15.9
IMC97	50	1.25	16.0
NA81	42	1.49	16.0
ICS43	38	1.64	16.1
CRU51	44	1.41	16.2
CRU38	49	1.26	16.2
Silecia8	44	1.40	16.2
CRU73	50	1.23	16.3
CRU35	43	1.43	16.3
LCTEEN261/S-4	43	1.41	16.5
AM1/85	39	1.54	16.7
IMC10	58	1.02	16.9
ICS5	43	1.37	17.0
JA5/35	43	1.37	17.0
ICS62	54	1.09	17.0
CRU126	46	1.27	17.1
B17/20	34	1.71	17.1
MOQ6/19	46	1.27	17.2
CRU2	43	1.35	17.2
JA5/31	45	1.29	17.2
AM2/91	40	1.45	17.2
ICS16	42	1.38	17.3
CRU111	35	1.64	17.4
ICS6	43	1.33	17.5
GS29	37	1.54	17.6
CRU104	39	1.46	17.6
LP3/40	38	1.49	17.7
Pound18	58	0.97	17.8
NA271	39	1.43	17.9
JA5/38	44	1.27	17.9
JA5/47	45	1.24	17.9
GS4	38	1.45	18.2
SLA68	45	1.22	18.2
EET58	34	1.61	18.3
SD1/6	57	0.96	18.3
JA1/17	44	1.24	18.3
JA3/35	43	1.26	18.5
JA5/24	44	1.23	18.5
CL27/50	38	1.41	18.7
ICS75	38	1.41	18.7

Table 2 (cont.). Accessions with favourable pod and bean characteristics

Accession	Bean number	Cotyledon weight	Pod index
IMC3	45	1.19	18.7
NA71	45	1.19	18.7
CRU129	42	1.27	18.8
IMC33	52	1.02	18.9
IMC65	50	1.06	18.9
AM2/43	37	1.43	18.9
JA1/1	40	1.32	18.9
B6/8	41	1.29	18.9
CLM114	44	1.20	18.9
CRU133	39	1.35	19.0
ICS111	36	1.46	19.0
LP1/40	39	1.37	19.0
Matina1/7	36	1.46	19.0
Pound7/B	50	1.05	19.1
EET397	41	1.28	19.1
JA1/10	43	1.22	19.1
JA5/25	43	1.22	19.1
IMC18 (Field 6 B)	49	1.07	19.1
JA10/54	39	1.34	19.1
CL10/5	39	1.33	19.3
CRU57	41	1.26	19.3
UF29	45	1.15	19.3
JA3/4	41	1.26	19.4
IMC71	56	0.92	19.4
SJ2/22	44	1.17	19.4
AM2/82	45	1.14	19.5
JA10/16	42	1.22	19.5
PA71	43	1.19	19.5
ICS63	39	1.31	19.6
ICS85	41	1.24	19.7
CRU137	40	1.27	19.7
IMC27	54	0.94	19.7
JA5/5	47	1.08	19.7
TRD45	36	1.41	19.7
JA10/4	43	1.18	19.7
B9/10-25	46	1.10	19.8
EET400	42	1.20	19.8
ICS8	40	1.26	19.8
ICS1	39	1.29	19.9
IMC61	57	0.88	19.9
FSC13	46	1.09	19.9
CC9	41	1.22	20.0
JA1/9	41	1.22	20.0

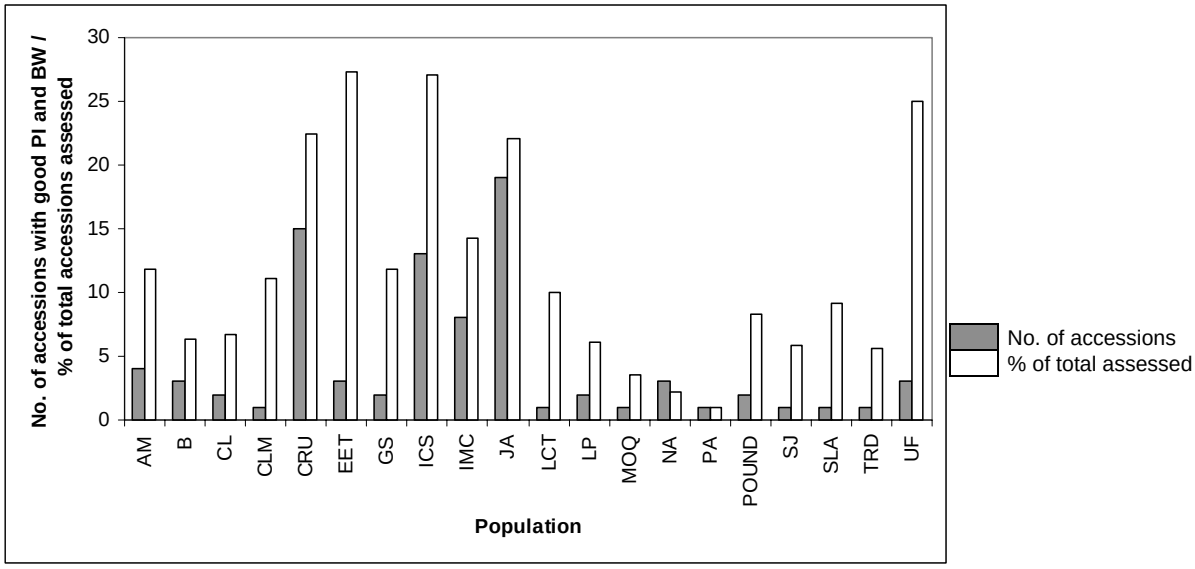

Fig. 1. Distribution of accessions with favourable bean weight and pod index for populations with more than nine accessions assessed.

Discussion

Some Accession Groups will be of particular interest to breeders due to their significantly superior agronomic traits. Bekele *et al*. (2006) observed improved mean values for cotyledon (and bean) weight and pod index in cultivated compared to wild germplasm, and this could reflect the impact of selection for characters of economic interest, and the results of hybridization. There has been success in breeding and selection for lower pod index. The occurrence of the most favourable pod index values in genotypes with either high bean number and moderate individual cotyledon weight (≈0.9 g) or large individual cotyledon weight and moderate bean number (≈40) or both large bean weight and number confirms the earlier finding that while dry cocoa production per pod is mainly dependent on bean number, average bean weight also plays an important role (Eskes *et al*. 1977; Engels 1983).

This apparent improvement in some of the components of yield of cultivated germplasm appears to be accompanied by an adaptive feature of the breeding system, particularly in the Refractarios: this is a longer style that may be more accessible to the pollinators (Bekele *et al*. 2006).

In the studies of Bekele *et al*. (2004, 2006), considerable phenotypic variation (high coefficients of variation and Shannon Weaver Diversity Index values) was observed in the germplasm (Table 3), and the main Accession Groups of cocoa were clearly separated. Recognized genetic groups (Trinitario and Forastero) were discriminated by several quantitative traits including sepal length, cotyledon weight, length and width, and pod index. Principal Component Analysis differentiated among 14 Accession Groups according to geographic origin and genetic grouping. Cotyledon weight, length and number, as well as pedicel column colour, mature pod ridge colour, sepal length, pod basal constriction and surface texture, and ovule number accounted for most of the variation recorded.

Table 3. Variances and Shannon Weaver Diversity Index values associated with each descriptor assessed for two samples of germplasm studied at the ICG,T (*Source: Bekele* et al. *2004)*

Descriptor	CFC Project Collection (85 accessions)		Larger sample (966 accessions)	
	Mean	Variances	Mean	Variances
Sepal length (cm)	7.56	0.89	7.59	0.76
Ligule width (mm)	2.45	0.1	2.44	0.09
Ovule number	43.1	34.0	43.7	30.0
Style length (mm)	2.25	0.08	2.25	0.11
Bean number	38.4	37.7	39.5	33.9
Cotyledon weight (g)	1.02	0.03	0.97	0.76
Cotyledon length (cm)	2.19	0.03	2.16	0.04
Cotyledon width (cm)	1.23	0.014	1.21	0.015
Pod length (cm)	16.3	2.95	15.9	3.38
Pod width (cm)	8.2	0.54	8.1	0.56
Wet bean weight (total) (g)	57.6	213.0	56.5	175.3
Pod index	26.9	39.1	27.8	50.5

Descriptor	CFC Project Collection (85 accessions)	Larger sample (966 accessions)
	Shannon Weaver Diversity Index	Shannon Weaver Diversity Index
Ligule colour	0.37	0.50
Filament colour	0.55	0.67
Pedicel colour	0.34	0.36
Mature pod colour	0.32	0.29
Pod shape	0.39	0.42
Husk hardness	0.25	0.84
Pod basal constriction	0.53	0.51
Pod apex form	0.64	0.67
Pod surface texture	0.46	0.44
Pod furrow disposition	0.14	0.07
Pod furrow separation	0.33	0.35
Cotyledon colour	0.29	0.37
Cotyledon shape	0.37	0.42

Conclusion

The value of morphological data generated at CRU has been demonstrated by their use as selection criteria for promising accessions, which have been included in the Germplasm Enhancement Programme (Iwaro *et al*. 2005) and in the CFC/ICCO/IPGRI Project Collection (Sounigo *et al*. 2005). The available body of morphological data as well as some photographs have been incorporated into CRU's database of the ICG,T, and are periodically submitted to the International Cocoa Germplasm Database.

There were serious limitations in the availability of material for study during the last six months of the project. This was possibly partly due to the severe drought in Trinidad in the recent past. It must be noted that without proper maintenance of the Genebank, the evaluation and utilization of the resources conserved therein are severely constrained. Effective irrigation is necessary for fruit production. Therefore, adequate funding for conservation of cocoa genetic resources must be assured.

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Description of cocoa clones proposed for the "CFC/ICCO/IPGRI Project Collection"

O. Sounigo[1], F.L. Bekele[2], A.D. Iwaro[2], J.-M. Thévenin[1], G. Bidaisee[2], R. Umaharan[2], A. Sankar[2], D. Sukha[2], L. Motilal[1], D.R. Butler[2] and A.B. Eskes[3]

[1] CIRAD-CP, TA 80/02, Avenue Agropolis, 34398 Montpellier cedex 5, France

[2] Cocoa Research Unit (CRU), University of the West Indies (UWI), St. Augustine, Trinidad and Tobago

[3] IPGRI/CIRAD, c/o INIBAP, Parc Scientifique Agropolis II, 34397 Montpellier cedex 5, France

Abstract

One important activity of the CFC/ICCO/IPGRI project entitled *"Cocoa Germplasm Utilization and Conservation: a Global Approach"* was the choice of approximately 100 cocoa clones to be distributed to all participating countries. The purpose was to help cocoa breeders worldwide to introduce into their research stations a collection enriched in sources of favourable alleles for several traits, such as resistance to several diseases (black pod (*Phytophthora* pod rot or Ppr) and witches' broom diseases, moniliasis and *Ceratocystis fimbriata*) and bean and pod characteristics (mean weight of one bean, fat content, pod index). The data used to establish the choice of 112 clones were obtained at the Cocoa Research Unit (CRU), in Trinidad, but also from other research institutes, from the International Cocoa Germplasm Database (ICGD) (University of Reading), or by informal contacts with researchers from several countries.

In addition, the choice of the clones was made with the aim of including a large amount of genetic diversity. This was ensured by choosing clones from a large number of populations, and by the use of molecular markers. Most of the clones were selected in the International Cocoa Genebank, Trinidad (ICG,T). The selected clones have been sent to the Reading International Cocoa Quarantine, from where they will be distributed to the research institutes. A catalogue with a detailed description of the selected clones has been prepared and this catalogue has been distributed to every participating institute on a CD-ROM.

Introduction

Around the world, cocoa breeding programmes are usually based on the use of rather limited sources of genetic diversity, generally combining the use of local or previously introduced varieties with that of more recently introduced genotypes. These last ones are usually issued from a limited number of collecting expeditions or selections. The fact that the wild populations resulting from collecting expeditions are usually represented by low numbers of genotypes in national cocoa collections is another source of limitation (Lockwood and End 1993). In order to help cocoa breeders enlarge the genetic base for their breeding programmes, one of the activities of the CFC/ICCO/IPGRI project entitled *"Cocoa Germplasm Utilization and Conservation: a Global Approach"* has been the definition of a "Project Collection", consisting of the selection of around 100 clones which would represent a high level of diversity but which would also be a rich source of favourable agronomic traits: mainly resistance to diseases but also good bean and pod characteristics.

This paper gives information on the strategy used to select the Project Collection as well as on the main characteristics of the clones that it contains.

Materials and methods

A search for potentially useful clones, from diverse geographic origins, was performed among:

- Material present in the ICG,T in which more than 1000 clones have been evaluated for different traits and characterized by the use of biochemical and molecular markers. These clones belong to a large number of populations of wild cocoa, mainly from Peru and Ecuador but also from other regions (French Guiana and Colombia). They also belong to selections of cultivated cocoa from several countries (Ecuador, Trinidad, Costa Rica and Brazil).
- Material present in other genebanks. Information was obtained from the International Cocoa Germplasm Database (ICGD) (Wadsworth *et al*. 1997), by reading the CFC/ICCO/IPGRI project reports and through informal contacts with cocoa researchers worldwide.

Details about analyses performed on the clones of the ICG,T at the Cocoa Research Unit (CRU) are given in Table 1.

Table 1. Details on the observations made at CRU on the cocoa clones of the ICG,T

Analysis	Details	Protocol	References
Genetic diversity	Isozyme electrophoresis	6 loci	Sounigo *et al.* 1997 Sounigo *et al.* 2001
	RAPD	30 markers from 13 primers	Sounigo *et al.* 2002
Pod and bean characterisics	Mean peeled and dried bean weight		Bekele *et al.* 1994 Bekele and Bekele 1998
	Pod index	Number of pods required to obtain 1 kg of dried cotyledons	Bekele *et al.* 1999
	Fat content (%)	Use of Soxhlet extraction technique	Khan 1997
Resistance to Ppr	Inoculation test	Performed on detached pods	Iwaro *et al.* 2000
	Rotten pods in the field (%)	Monthly observations during a period of 1 to 3 years	Latchman *et al.* 2000
Resistance to witches' broom disease	Infected shoots in the field (%)	Precise counting of brooms on a limited number of shoots three times a year during a period of 1 to 3 years	Latchman *et al.* 2000
	Infected shoots in the field (%)	Quick estimation of the number of brooms on the whole tree. Only one round.	Sounigo, unpublished
	Infected pods in the field (%)	Monthly observations during a period of 1 to 3 years	Latchman *et al.* 2000
Yield	Number of healthy ripe pods	Monthly observations during a period of 1 to 3 years	Latchman *et al.* 2000

Data from the ICGD and through informal contacts with cocoa researchers were obtained on some of the traits described in Table 1 and also on other traits such as resistance to diseases (vascular streak dieback (VSD), moniliasis and dieback caused by *Ceratocystis*), self-compatibility, low vigour, good yield.

In a previous study, genetic diversity assessments on clones of the ICG,T using random amplified polymorphic DNA (RAPD) and isozyme electrophoresis (IE) showed Shannon index differentiation indices between "populations" (genotypes issued from the same geographical area) of 0.35 and 0.28, respectively. This allowed us to adopt a sampling strategy based on the stratification according to "populations".

The RAPD and IE techniques were then used in order to:

- choose the level of representation of each population according to its level of diversity, calculated by Shannon indexes; and
- ensure that the clones included in the Project Collection cover a large part of the genetic diversity of the ICG,T, by comparing results of multivariate analyses.

Data on agronomic traits were used to perform positive and/or negative selection, with different levels of selection pressure. A positive selection made on one trait means that a good value for this trait increases the chances for a clone of being included in the Project Collection. A negative selection on one trait means that a poor value for this trait decreases the chances for a clone of being included in the Project Collection. The type and intensity of selection are indicated for every trait in Table 2.

Table 2. Type and intensity of selection for each trait

Trait	Positive selection	Negative selection
Resistance to Ppr	strong	strong
Resistance to witches' broom disease	fair to strong	moderate
Mean weight of one peeled dried bean	moderate	strong
Fat content	moderate	no
Pod index	moderate	no
Resistance to moniliasis	strong	no
Resistance to vascular streak dieback	fair	no
Resistance to *Ceratocystis fimbriata*	fair	no
Yield	strong	no
Self-compatibility	fair (ICS clones)	no

The highest selection pressure was placed on resistance to diseases since it is a major issue for cocoa breeding, especially on resistance to Ppr, since it is present in all producing countries. In the case of artificial pod inoculation using spores of *Phytophthora*, only the clones showing the most severe symptoms were discarded (see threshold values in Table 3).

Table 3. Threshold values for resistance and susceptibility to Ppr and witches' broom disease

Trait evaluated	Inoculation	Potentially resistant Scores 1 to 6	Susceptible Scores 7 and 8
Resistance to Ppr	Rotten pods (%)	≤25% in year 1 and year 2 ≤40% in year 3	>25% in year 1 and year 2 >40% in year 3
Resistance to witches' broom disease	Infected shoots (%)	≤2.5% (precise counting on a delimited area of the tree) ≤10 brooms (quick counting on the whole tree)	>2.5% (precise counting on a delimited area of the tree) >10 brooms (quick counting on the whole tree)
	Infected pods (%)	≤25%	>25%

Indeed, we preferred to be less restrictive in order to maximize the diversity of resistance alleles captured in the Project Collection. In the field, trees were observed for a period of one, two or three years, by monthly harvests followed by counting of healthy pods, pods infected by *Phytophthora* and pods infected by *Crinipellis perniciosa* (recently renamed *Moniliophthora perniciosa)*. Every year, the cumulative percentage of rotten pods was estimated for each clone and the mean value was calculated for all the clones analyzed in the ICG,T. According to these percentages of rotten pods, clones were classified as susceptible or potentially resistant (see threshold values in Table 3). When both types of information were available, only the clones found potentially resistant to black pod disease (*Phytophthora* pod rot or Ppr)

after both inoculation experiments and field observations were included in the Project Collection.

The levels of witches' broom attack on shoots were assessed by two different methods:

- 697 clones were assessed by a precise counting of the brooms on a delimited area of the trees, and percentages of attacked shoots were estimated. These counts were performed three times a year, for a period from one to three years;
- 476 clones were assessed by one single round of observation, consisting of a quick count of brooms on the whole tree.

According to these values, clones were classified as susceptible or potentially resistant (see threshold values in Table 3).

In the case of resistance to moniliasis, a strong level of positive selection was adopted because the sources of resistance to this disease are very scarce (Phillips-Mora 1999).

The mean weight of one peeled and dried bean was only used as a negative factor of selection, in order to prevent the inclusion of clones with small beans in the Project Collection.

Yield was measured by counting healthy mature pods on the same trees used for evaluation of rotten pods. The most promising clones for this trait were included in the Project Collection, since Lockwood and Pang (1993) found a positive correlation between the values as clone and as progenitor for this trait.

Self-compatibility was used as a positive factor in the case of the ICS clones to prevent problems of cross-incompatibility (Cope 1962).

Results

Level of genetic diversity

The list of the 112 clones selected for the Project Collection is given in Table 4, while Table 5 gives details about the populations represented by these clones.

Most of these clones (88) were selected in the ICG,T but 24 of them (represented in bold in Table 4) were sourced from other collections, where they had shown their high potential.

The number of clones represented in each "population" depends on its level of genetic diversity, measured in a previous study on a large sample of the ICG,T, using IE and RAPD, but also on the number of clones with favourable traits belonging to the population. The CAM population from French Guiana is represented by a relatively large number of clones, despite its rather low level of diversity, because molecular analyses using RAPD showed that this population is very distinct from all the other populations (Sounigo *et al.* 2002). The LCT EEN "population" is also represented by a large number of clones, since it presented a high diversity index when studied by both IE and RAPD techniques, and also because it was found to be very different from the wild populations of Peru (AMAZ, IMC, NA, MO, PA, Pound and SCA) in a study based on SSR (simple sequence repeats) (Motamayor, unpublished).

Both wild and cultivated clones are included in this collection, the first ones being expected to provide sources of resistance to disease, while the cultivated ones are expected to provide sources of favourable alleles for pod and bean characteristics.

Table 4. List of the clones included in the Project Collection. Clones indicated in bold do not originate from the ICG,T. Names are according to the usage in the International Cocoa Germplasm Database (ICGD)

AM 1/57 [POU]	**EET 75 [ECU]**	LCT EEN 162/S1010	PA 13 [PER]
AM 2/19 [POU]	**EET 233 [ECU]**	**LCT EEN 163/A**	PA 39 [PER]
AMAZ 1/5 [CHA]	**EET 289 [ECU]**	LCT EEN 212/S4	**PA 70 [PER]**
AMAZ 5/2 [CHA]	**EET 333 [ECU]** (SILECIA 5)	**LCT EEN 241**	PA 120 [PER]
AMAZ 6/3 [CHA]	EET 399 [ECU]	LCT EEN 261/S4	PA 121 [PER]
B 5/7 [POU]	FSC 13	LCT EEN280	PA 124 [PER]
B 6/3 [POU]	**GU 133/C**	LCT EEN 302	PA 126 [PER]
B 9/10-25 [POU]	GU 175/P	LP 3/4 [POU]	PA 169 [PER]
B 9/10-32 [POU]	GU 241/P	LP 3/15 [POU]	PA 279 [PER]
B 12/1 [POU]	**GU 255/V**	LX 31	PA 299 [PER]
B 13/5 [POU]	GU 261/P	LX 43	PA 303 [PER]
CC 71	ICS 35	**MA 12 [BRA]**	**POUND 7/B [POU]**
CC 137	ICS 43	MAN 15/2 [BRA]	POUND 15/A [POU]
CCN 10	ICS 55	MATINA 1/7	RUQ 233 (MIS_GBRUQ_MO20)
CCN 51	ICS 63	MO 4	**SC 1**
CL 10/5	ICS 76	MO 9	SCA 9
CL 10/27	ICS 83	MO 109	SCA 10
CL 19/10	ICS 95	**MOQ 6/19**	SCA 24
COCA 3348/44 [CHA]	IMC 20	MOQ 6/95	SJ 1/40 [POU]
COCA 3370/5 [CHA]	IMC 31	MOQ 6/99	SLC 4
CRINKLE LEAF	**IMC 47**	**NA 33**	**SPA 7**
CRU 12	IMC 50	NA 184	SPEC 41/6-18
CRU 80	IMC 94	NA 232	TRD 45
CRU 89	JA 5/5 [POU]	NA 399	TRD 85
CRU 104	**LCT EEN 37/I**	NA 702	TRD 109
CRU 126	LCT EEN 46	NA 710	**UF 273**
CRUZ 7/14	LCT EEN 62/S4	NA 807	UF 613
EET 59 [ECU]	LCT EEN68/S2	NA 916	**UF 712**

Table 5. Details on the populations represented in the Project Collection

Population	No. of clones	Origin	Type	Shannon diversity index	
				IE*	RAPD**
AM	2	Ecuador	cultivated	0.60	0.31
AMAZ	3	Peru	cultivated	0.43	0.35
B	5	Ecuador	cultivated	0.59	0.24
CC	1	Costa Rica	cultivated		
CCN	2	Ecuador	cultivated		
CL	2	Ecuador	cultivated	0.62	0.38
COCA	2	Ecuador	wild		
CRU	6	Ecuador and Peru	cultivated and wild		
CRUZ	1	Brazil	wild		
EET	5	Ecuador	cultivated		
FSC	1	Unknown			
CAM	5	French Guiana	wild	0.21	0.21
ICS	9	Trinidad	cultivated	0.66	0.31
IMC	5	Peru	wild	0.43	0.36
JA	1	Ecuador	cultivated	0.54	0.24
LCTEEN	15	Ecuador	wild	0.70	0.42
LP	2	Ecuador	cultivated	0.53	
LX	2	Ecuador	cultivated	0.52	
MA	1	Brazil	cultivated		
MAN	1	Brazil	cultivated		
Matina	1	Costa Rica	cultivated		
MO	4	Peru	wild	0.65	0.36
MOQ	3	Ecuador	cultivated	0.62	0.36
NA	8	Peru	wild	0.50	0.31
PA	11	Peru	wild	0.41	0.32
Pound	4	Peru	wild	0.47	0.36
SC	1	Colombia	cultivated	0.60	
SCA	3	Peru	wild	0.55	0.36
SJ	1	Ecuador	cultivated	0.43	
SL	1	Ecuador	cultivated	0.68	
SPEC	2	Colombia	cultivated and wild		
TRD	4	Trinidad	cultivated	0.61	0.38
UF	2	Costa Rica	cultivated	0.60	0.31

* IE = isozyme electrophoresis

** RAPD = random amplified polymorphic DNA

Figures 1 and 2 compare the levels of genetic diversity observed in the Project Collection and in a large subsample from the ICG,T, using IE and RAPD respectively. They indicate that the Project Collection covers a large part of the diversity of the ICG,T.

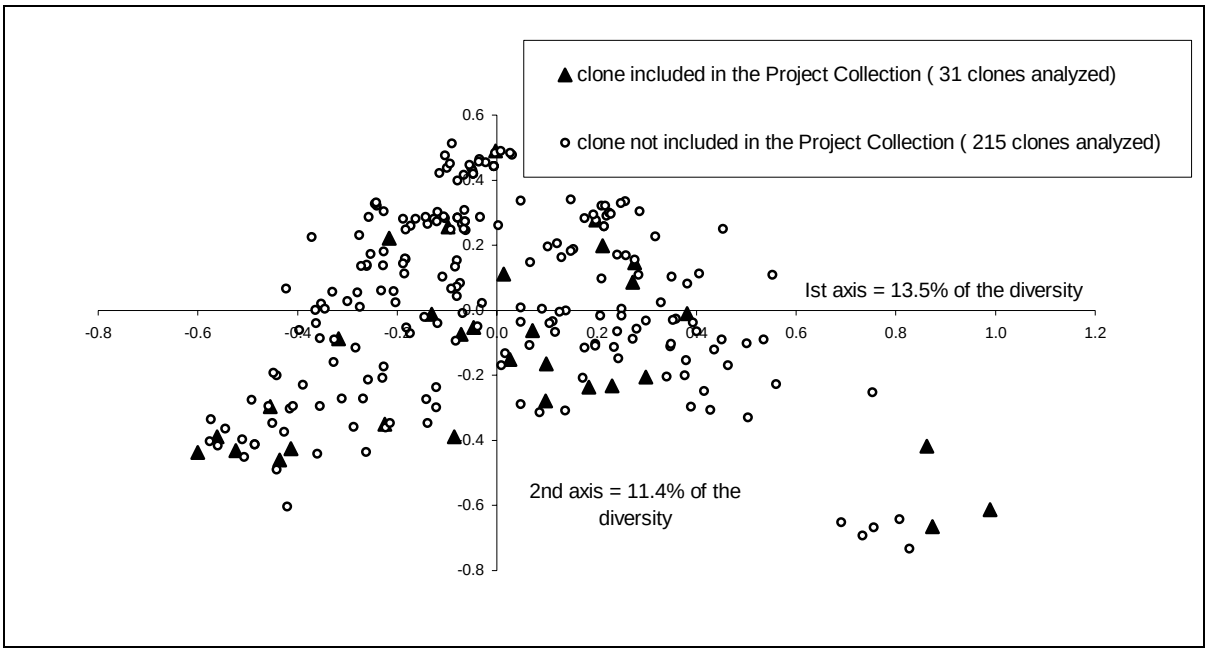

Fig. 1. Plane defined by the first two axes of a Factorial Analysis of Correspondences performed on RAPD data.

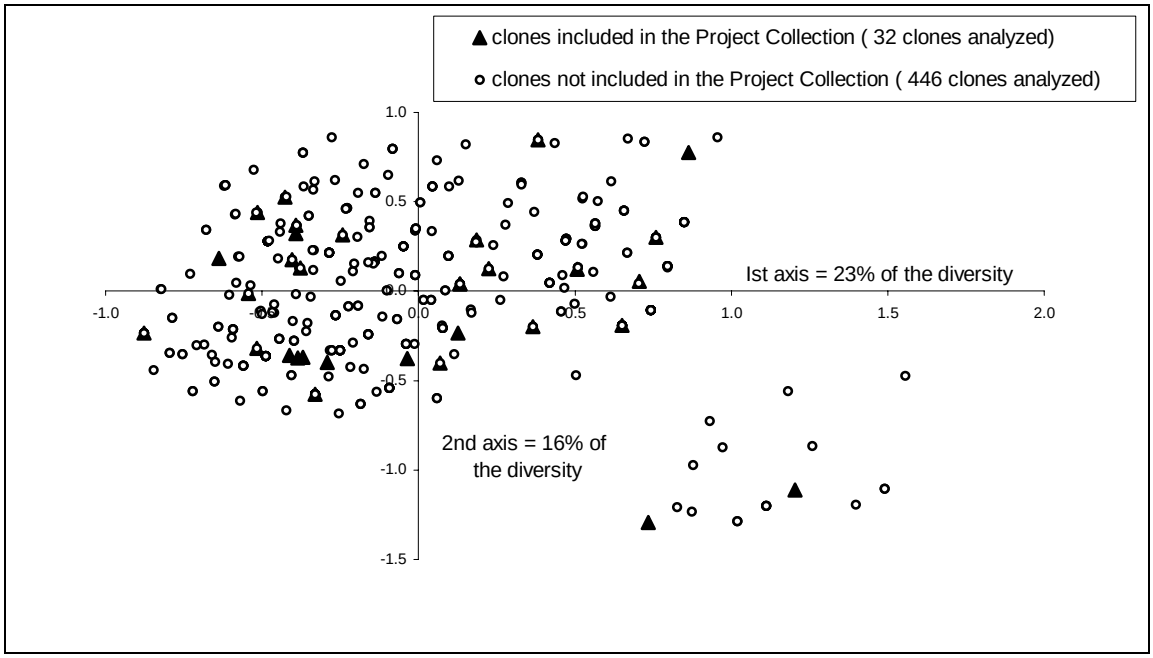

Fig. 2. Plane defined by the first two axes of a Factorial Analysis of Correspondences performed on IE data.

Sources of favourable traits

The Project Collection should be a major source of favourable alleles for resistance to Ppr because of strong selection for this trait, both positive and negative. Indeed, most of the clones can be considered as potential sources of resistance to this disease, the most promising clones being those with a high level of resistance when tested using a pod inoculation test (score of 5 or lower) and showing a low percentage of naturally infected pods in the field (lower than the mean value for the same period in the same plot).

A lower number of clones with resistance to witches' broom was observed because of a weaker negative selection level for this trait. Even so, about 40% of the clones can be considered to be potential sources of resistance to this disease. The most promising clones for this trait are those found resistant in Ecuador or in Brazil, under higher conditions of parasitic pressure than in Trinidad.

Due to the scarcity of sources of resistance to moniliasis, most of the clones that are known to be potentially resistant to this disease were included in the Project Collection. Despite the relatively low level of positive selection for larger beans, it appears that about 50% of the clones in the Project Collection present this favourable trait. Again, despite a low level of positive selection for low pod index, the Project Collection still contains 20 clones with this desirable trait. Similarly, the low level of selection for high fat content resulted in the inclusion of nine promising clones for this trait. Nine promising clones for high yield potential were also included in the Project Collection. SCA24 and Crinkle Leaf were included because of their very low vigour, as potential sources for dwarfing rootstocks or in breeding for trees with a small canopy.

The most promising clones for the above traits, according to the current information, are shown in Table 6.

Table 6. Clones of the Project Collection promising for Ppr resistance (6A) and for other desirable traits (6B)

A. Promising clones for resistance to Ppr*							
AM 1/57	CC 71	CRUZ 7/14	IMC 94	MOQ 109	NA 710	PA 121	P 15/A
AMAZ 6/3	CL 10/5	FSC 13	LP 3/4	MOQ 6/19	NA 807	PA 124	SCA 10
B 5/7	CL 19/10	GU 133/C	LP 3/15	MOQ 6/95	NA 916	PA 126	SJ 1/40
B 6/3	COCA 3370/5	ICS 63	LX 31	MOQ 6/99	PA 13	PA 169	SLC 4
B 9/10-25	CRU 12	IMC 20	LX 43	NA 184	PA 39	PA 279	SPEC 41/6-18
B 9/10-32	CRU 80	IMC 47	MA 12	NA 232	PA 70	PA 299	TRD 85
B 13/5	CRU 89	IMC 50	MO 4	NA 399	PA 120	P 7/B	TRD 109

* Based mainly on combination of scores of 5 or lower in the detached pod inoculation test and field observations in Trinidad (R or MR)

B. Promising clones for other traits								
Resistance to			Bean size (medium to large)		Low pod index		High fat content	Yield
Witches' broom**		Monilia						
AMAZ 1/5 (B)	JA 5/5 (T)	CC 137	B 9/10-32	GU 175/P	AM 1/57	ICS 63	CRU 80	B 5/7
B 12/1(T)	MATINA 1/7 (T)	EET 75	CC 71	GU 261/P	CL 10/5	ICS 83	EET 399	B 12/1
CCN 10 (B)	LCT EEN37/I (E)	EET 233	CCN 10	ICS 63	CRU 104	MATINA 1/7	MOQ 6/95	CC 137
CCN 51 (E)	LCT EEN46 (E)	EET 289	CCN 51	ICS 83	CRU 111	MOQ 6/19	NA 702	CCN 51
COCA 3370/5 (B)	LCT EEN 162/S1010 (B)	EET 399	CL 10/5	LCT EEN 261/S4	CRU 12	PA 73	PA 169	CRU 12
CL 10/5 (T)	NA 399 (T)	ICS 95	CRU 104	MATINA 1/7	CRU 126	POUND 7/B	PA 279	MA 12
EET 233 (E)	NA 807 (T)	PA 169	CRU 111	MOQ 6/19	CRU 89	TRD 109	PA 299	MAN 15/2
EET 333 (E)	PA 120 (T)	UF 273	CRU 126	PA 73	FSC 13	TRD 45	SCA 9	NA 702
EET 399 (B,E,T)	PA 303 (T)	UF 613	CRU 89	SC 1			SPA 7	PA 13
GU 241/P (T)	SJ 1/40 (T)	UF 712	EET 333	TRD 45				
IMC 47 (T,E)	SLC 4 (T)		EET 399	UF 273				
			EET 59	UF 613				

**Based on field observations in Trinidad (T), Brazil (B) and Ecuador (E)

Availability in Reading Quarantine

About 30 of the clones of the Project Collection were already available for distribution at the Reading quarantine facility in 2004. The others will be sent to Reading over the next few years, mainly from Trinidad but also from other sources (e.g. Costa Rica, Brazil and Ecuador).

More detailed information on the Project Collection clones

The information available in 2004 about the Project Collection clones is reproduced in Annex 1 (pp. 77-81) and also in an electronic catalogue that is part of the CD-ROM that was distributed to all participating institutes with the presentations of the Closing Workshop of the project (Reading, April 2004). The same catalogue can be consulted at the INGENIC Web site (http://ingenic.cas.psu.edu).

The catalogue is composed of several Excel files. The file to open is called "Project catalogue.xls" and is a simple Excel worksheet with a table listing the clones of the Project Collection and giving a brief description of them. Clicking once on the desired clone name opens another Excel file composed of several worksheets, each of them giving details on the clone: the first one with pictures of the pods, the second one with detailed information on resistance to disease, the third one with information on pod and bean characteristics and the fifth one with information on yield and self-compatibility. In some cases, only some of these sheets are present because a complete dataset is not available for these accessions. In the cases of SCA24 and Crinkle Leaf, another sheet gives information on the vigour of these clones.

Discussion

Results obtained from molecular analyses indicate that sampling among a large number of cocoa populations, representing a wide range of geographical origins, allowed a good level of genetic diversity to be maintained even when selecting for several useful traits. The maintenance of a good level of diversity was rendered easier by the fact that sources of resistance to Ppr, the main trait used for both positive and negative selection, are not restricted to a few populations, but are distributed in a rather large range of them (Iwaro *et al.* 2001).

The Project Collection is expected to be a major source of favourable alleles for several desirable traits and some of its clones bring together several favourable traits. Such clones should be tested as progenitors in order to obtain valuable progenies after one single cycle of crosses. This is the case with IMC47 which shows resistance to both Ppr and witches' broom diseases as well as good yield potential. Another example is CL10/5, which shows resistance to Ppr and good pod and bean characteristics. UF613 is one of the rare sources of resistance to moniliasis and produces large beans. CCN10 is a source of resistance to witches' broom disease combined with large beans.

As a strategy for introduction of the Project Collection, it is recommended that priority be given to clones with resistance to prevailing diseases in the country (e.g. Ppr resistance in Africa) but also to diseases which are not present in the country to avoid being completely unarmed if they appear (preventive breeding). For example, it seems wise to include sources of resistance to witches' broom disease and moniliasis in the breeding programmes of the African and Asian countries.

Since new germplasm is continuously collected and evaluated, such an activity should continue and the Project Collection should be enriched every year with promising new clones. This is the reason why the catalogue describing theses clones is in an open form, allowing everyone to complete it as soon new information is available and new clones are included in the Project Collection.

Conclusion

Using a rather pragmatic approach, it was possible to select a subset of 116 cocoa clones combining favourable traits and genetic diversity, using several types of information. This exercise was made possible by the collaboration between several researchers from CRU: breeders, plant pathologists, molecular biologists, botanists and chemists, but also by the use of the ICGD and through informal contacts with cocoa researchers worldwide. It is expected that this Project Collection will be of significant use in cocoa breeding worldwide. The collection should be periodically updated by adding new sources of potentially valuable materials in the future.

Acknowledgements

This activity was a part of the project entitled "*Cocoa Germplasm Utilization and Conservation: a Global Approach*". This project, developed under the aegis of the International Cocoa Organization (ICCO, London) has been partly funded by the Common Fund for Commodities (CFC), an intergovernmental organization headquartered in Amsterdam, The Netherlands.

In addition to the CFC and the ICCO, the authors would also like to thank the International Plant Genetic Resources Institute (IPGRI) for the management of this project.

Also, the Biscuit, Cake, Chocolate and Confectionery Association (BCCCA, London) and the Government of Trinidad and Tobago should be thanked for their financial support to the research activities carried out in CRU.

The authors do not forget all the CRU staff involved in this activity and also thank colleagues from other research institutes who provided information, through the channel of the ICGD or other documents, or simply by e-mails.

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Annex 1. Proposed shortlist of accessions to be part of the "CFC/ICCO/IPGRI Project Collection" and traits observed. Naming is according to the International Cocoa Germplasm Database (for sources of information see legend (1) p. 81)

Clone	Source (2)	BP inoculation (3)	BP Iwaro rating (3)	BP field (3)	WB shoots (3)	WB pods (3)	Cotyledon weight (4)	Dry bean weight (4)	Pod Index (5)	Pod hardness	% Fat	Miscellaneous (6)
AM 1/57 [POU]	TTO	MR	5		R		0.9		vl			
AM 2/19 [POU]	TTO	MS	6	R	R	R	1.14		l			
AMAZ 1/5 [CHA]	BRA	MS **MR (CIV)**	6		S **R (BRA) MS (ECU)**		1.14		l	hard		Clone that is often wrongly identified as AMAZ15
AMAZ 5/2 [CHA]	TTO	**R (CIV)**			R							
AMAZ 6/3 [CHA]	TTO	MR	5	R	R	MR	0.88		h m	hard		
B 5/7 [POU]	TTO	MR	4	MR	R	R	1.15		h		50.6	
B 6/3 [POU]	TTO	R	3		R		1.1		h			
B 9/10-25 [POU]	TTO	MR	5	MR	R	R	1.01		vl		54.4	
B 9/10-32 [POU]	TTO	MR	4				1.2		l		52.4	
B 12/1 [POU]	TTO	MS	6	R	R	R	0.83		vh		51.5	
B 13/5 [POU]	TTO	MR	5	R	R	MR	0.91		m			
CC 71	TTO	MR **MR (CRI)**	4	MR	R	MS		**1.09 (MYS) 1.22 (MYS)**	m		**53.9 (MYS) 54.6 (MYS)**	
CC 137	CRI	MR (CRI)										**R to Mon (CRI)**
CCN 10	BRA				**R (BRA)**			**2.1 (PER) 1.33 (MYS) 1.44 (MYS)**	**vl (PER) l (MYS)**			**S to VSD (MYS)**
CCN 51	ECU				**MR (BRA and ECU)**	MR (BRA)		**1.5 (PER)**	**vl (PER)**			**High yield (ECU)**
CL 10/5	TTO	R	1	MR	R	R	1.33		vl		47.6	
CL 10/27	TTO	MR	5		R		1.09		m			
CL 19/10	TTO	R	3	MR	**R**	MR	0.85		vh			
COCA 3348/44 [CHA]	TTO				R		0.9		l			
COCA 3370/5 [CHA]	TTO	R	3		**R (BRA)**		1.05		m			
CRINKLE LEAF	GHA											**Dwarf (GHA)**
CRU 12	TTO	R	1	MR	R	MR	1.05		m		52.4	
CRU 80	TTO	R	2	MR	R	R	0.87		h		56.3	
CRU 89	TTO	R	2	MR	R	R	1.35		vl		51.3	
CRU 104	TTO	MS	6	R	R	R	1.46		vl			

Clone	Source (2)	BP inoculation (3)	BP Iwaro rating (3)	BP field (3)	WB shoots (3)	WB pods (3)	Cotyledon weight (4)	Dry bean weight (4)	Pod Index (5)	Pod hardness	% Fat	Miscellaneous (6)
CRU 126	TTO	MR	5		R		1.27		l			
CRUZ 7/14	TTO	R	2	MR	R		.89 1.07		m l	hard		
EET 59 [ECU]	TTO	R R (CRI) MR (CIV) MR (MEX)	2	MR	S S (BRA)	S	1.4	1.4	vl	hard		
EET 75 [ECU]	CRI	MR (CRI)					0.78 (CRI)		h (CRI)			R to Mon (CRI), High yield (CRI), SI (CRI)
EET 233 [ECU]	ECU				R (ECU)							R to Mon (ECU), S to Cer (ECU)
EET 289 [ECU]	ECU											R to Mon (ECU)
EET 333 [ECU] (SILECIA 5)	ECU	MR (CRI)			R (ECU)		1.17 (CRI) 2.4 (CRI)	1.18	m			
EET 399 [ECU]	TTO	MR MR (CRI) R (MEX)	5		R R (ECU) R (BRA)		1.27 1.01 (CRI) 1.36 (CRI)	1.12 (MYS) 1.15 (MYS)	l m (CRI) m (MYS)	hard	58.3	R to Cer (CRI), R to VSD (MYS)
FSC 13	TTO	R	2				1.09		vl			
GU 133/C	FRA	R (CIV)										
GU 175/P	TTO	MR	5		R	R		1.31 (BRA)				
GU 241/P	TTO	MS	6	R	R	R						
GU 255/V	FRA	R (FRA)			R							
GU 261/P	TTO	MR			R MR (BRA)			1.47 (BRA)				
ICS 35	TTO	MR	5		R		1.06		h	medium		SI
ICS 43	TTO	MS MR (CMR)	6	S	S		1.64					
ICS 55	TTO	MS	6	S R (TGO)	R R (BRA)		1.00			soft		SC SC (CRI and (BRA), MR to Cer (CRI)
ICS 63	TTO	MR	5	R	R R (ECU)		1.34		vl			SI
ICS 76	TTO	MS	6	R	R	R						SC
ICS 83	TTO	MR	5				1.19		vl			SI
ICS 95	TTO	MS MS (CRI) MR (CMR)	6	S R (TGO)	MS MR (BRA) R (ECU)	S	1.15 0.69 (CRI) 1.00 (CRI)	0.9 (MYS) 0.94 (MYS) 1.1 (MYS)	l m (MYS) h (MYS) vh (CRI)	hard soft (CRI)	57.3	C SC (CRI, BRA and MYS) MR to Mon (CRI) MS to mirids (CIV) R to Cer (CRI)
IMC 20	TTO	R	1	MR	R	R	0.96		l	medium	55.8	

Clone	Source (2)	BP inoculation (3)	BP Iwaro rating (3)	BP field (3)	WB shoots (3)	WB pods (3)	Cotyledon weight (4)	Dry bean weight (4)	Pod Index (5)	Pod hardness	% Fat	Miscellaneous (6)
IMC 31	TTO			MR **MR (CIV)**	R	R	0.97		l	hard		**R to Cer (CRI)**
IMC 47	FRA	R **R (CIV)**	1	R **R (BRA)**	R	R	0.83 1.00	**0.79 (MYS)** **1.03 (MYS)**	m **m (MYS)**	medium	53.5	
IMC 50	TTO	R	2				0.95		l	hard		
IMC 94	TTO	R	2	MR	R	R	0.93		l	hard	54.8	
JA 5/5 [POU]	TTO	MS	6	R	R	R	1.08		vl	hard	52.4	
LCT EEN 37/I	USA				**R (ECU)**						52.5	
LCT EEN 46	ECU	**S (CIV)**			**R (ECU)**		0.7					This clone, distributed for the International Clone Trial, is apparently not the original clone from San Carlos, Ecuador.
LCT EEN 62/S4	TTO	MS	6	MR	MS	S	0.88		h			
LCT EEN 68/S2	TTO						0.88		m			
LCT EEN 162/S1010	TTO	MS	6	MR	MS **R (BRA)**	S	0.92		m			
LCT EEN 163/A	USA	MS	6		R							
LCT EEN 212/S4	TTO	MS	6		MS							
LCT EEN 241	USA						1.30					white
LCT EEN 261/S4	TTO	MS	6		R		1.41					
LCT EEN280	TTO	MR	5		R	R	1					
LCT EEN 302	TTO						1.05					white
LP 3/4 [POU]	TTO	R	2	R	R	R	1.14		m		50.9	
LP 3/15 [POU]	TTO	MR	4	MR	R	R	1.07		vh		53.4	
LX 31	TTO	R	2	R	R	S	0.9		m	hard		
LX 43	TTO	MR	4				1.11		h	hard		
MA 12 [BRA]	Reading	**S (CRI)**		**R (GHA, CMR)**	**MR (BRA)**		**0.83 (CRI)**	**2.59 (BRA)**	**vl (BRA)** **h (CRI)**		53.9	**SC (CRI)** **SI (BRA)**
MAN 15/2 [BRA]	TTO	**MR (FRA)**			**S (ECU)**							
MATINA 1/7	TTO	MS	6	MR	R	R	1.46	2.11	l		54.9	
MO 4	TTO	R	3		R		1.03		m			
MO 9	TTO	MR **MR (CIV)**		**MR (CIV)**	R		0.86 0.93		L	hard	54.2	
MO 109	TTO	MR	5				0.86		vh	hard		

Clone	Source (2)	BP inoculation (3)	BP Iwaro rating (3)	BP field (3)	WB shoots (3)	WB pods (3)	Cotyledon weight (4)	Dry bean weight (4)	Pod Index (5)	Pod hardness	% Fat	Miscellaneous (6)
MOQ 6/19	TTO	R	3		S		1.27		l			
MOQ 6/95	TTO	R	1		R		1.06		h		56.5	
MOQ 6/99	TTO	MR	4		R	R	1.02		l		52.6	
NA 33	MYS	S		**R (BRA)**	**R (BRA)**			**0.70 (MYS)** **0.78 (MYS)**	m		53.4	**Good yield (MYS)** **R to VSD (MYS)**
NA 184	TTO	R	2	R	R	MR	0.95		h			
NA 232	TTO	MR	4	MR	R	MR	1.11		m			
NA 399	TTO	R	1	MR	R	R	0.85		l		53.9	
NA 702	TTO	MR		MR	R	R	0.87		vh		57.2	
NA 710	TTO	R	3	MR	R	MR	0.89		h		54.8	
NA 807	TTO	MR	4	MR	R	R	0.86		h		54.5	
NA 916	TTO	MR	4				1.06		m	hard	54.7	
PA 13 [PER]	TTO	R	3	**MR (CIV, CRI)** **R (BRA)**	R		0.76 **1.05 (CRI)**	**0.9 (MYS)**	h			**High yield (CIV)**
PA 39 [PER]	TTO	MR	4	MR	R	R	0.97		m			
PA 70 [PER]	GBR	R, **R (GHA, MEX)**	3	MR	S	MR	0.91 0.99		l h	hard	55.8	
PA 120 [PER]	TTO	R	3	R	R	R	0.82		vh			**Low yield (TTO and PNG)**
PA 121 [PER]	TTO	MR **R (CIV, MEX)** **S (CRI)**	4	MR	R **MR (BRA)**	R	0.96 **0.94 (CRI)** **1.00 (CRI)**	**0.98 (MYS)** **1.04 (MYS)**	h **h (MYS)**	hard **soft (CRI)**		**S to Cer (CRI)** **S to VSD (MYS)**
PA 124 [PER]	TTO	R	2		R		0.86		m			
PA 126 [PER]	TTO	MR	5	MR	R	R	0.89		h	hard		
PA 169 [PER]	TTO	R	4	MR	R	MR	1.01 **1.07 (CRI)**		m		**56.1 (BR)**	**MR to Mon (CRI)**
PA 279 [PER]	TTO	R	3	MR	R	R	0.95		m	medium	56.8	
PA 299 [PER]	TTO	MR	4	R	R	MR	0.89 1.00		vh	hard	56	
PA 303 [PER]	TTO	MS	6	MR	R	R	0.98		h			**R to Cer (CRI)**
POUND 7/B [POU]	GBR	MR	4		R		1.05		vl	hard		Likely to be identical to P7
POUND 15/A [POU]	TTO	MR	5		MR		0.88		h			

Clone	Source (2)	BP inoculation (3)	BP Iwaro rating (3)	BP field (3)	WB shoots (3)	WB pods (3)	Cotyledon weight (4)	Dry bean weight (4)	Pod Index (5)	Pod hardness	% Fat	Miscellaneous (6)
RUQ 233 (MIS_GBRUQ_MO 20)	Reading	MS	6		R **R (BRA)**		0.98		h	hard		This is the "MO20" clone that was distributed for the International Clone Trial, but it is not the original MO20 type from Marper
SC 1	GBR	MS	6				1.29 **1.53 (COL)**		m	medium		
SCA 9	TTO	R **MR (CRI)**			S		1.08 **0.99 (CRI)**	**0.97 (MYS) 0.65 (MYS)**	l **vh (CRI)**	hard		
SCA 10	TTO	MR	5				1.11					
SCA 24	TTO	S	7	S	**R (ECU)**			small	vh			**dwarf**
SJ 1/40 [POU]	TTO	R	2	R	R	R	0.93		m	hard		
SLC 4	TTO	R	1	MR	R	MR	1.02		m	hard	48.8	
SPA 7	TTO	S	8		R		1.24		l		**55.4 (BRA)**	**R to Mon (CRI) SI (CRI and BRA)**
SPEC 41/6-18	TTO	MR	4	R	R	MR	0.72		vh	hard		
TRD 45	TTO	MS	6	R	R	R	1.41		vl	hard		
TRD 85	TTO	R	2			R	1.16		m			
TRD 109	TTO	MR	5	MR	R	R	1.16		vl			
UF 273	CRI	**MR (CRI)**					1.31					**SC (CRI) MR to Cer (CRI)**
UF 613	TTO	MS **MR (MEX) MR (CRI)**	6		MS		1.4	**1.11 (MYS) 1.20 (MYS)**	m	hard	56.3	**SI (CR, MAL, (BRA) R to Cer (CRI) MR to VSD (MYS)**
UF 712	CRI	**S (CRI)**						1.16	m			**R to Mon (CRI) SI (CRI)**

(1) Observations made by CRU, Trinidad are not in bold. Data from other sites are indicated in bold: BRA = Brazil, CIV = Côte d'Ivoire, CMR = Cameroon, COL = Colombia; CRI = Costa Rica, ECU = Ecuador, FRA = France, GHA = Ghana; MYS = Malaysia, MEX = Mexico, PER = Peru; PNG = Papua New Guinea; TGO = Togo; TTO = Trinidad and Tobago.

(2) Source = source collection for introduction into Reading of the accessions of the CFC Project Collection.
BRA = CEPEC, Brazil; CRI = CATIE, Costa Rica; ECU = INIAP, Ecuador; FRA = CIRAD, France; GHA = CRIG, Ghana; MYS = MCB, Malaysia; TTO = ICG, Trinidad and Tobago; Reading = University of Reading, GBR= Kew Gardens; USA = USDA, Miami.

(3) S = susceptible, MS = moderately susceptible, MR = moderately resistant, R = resistant.
Black pod (BP) reaction to artificial inoculation: in Côte d'Ivoire (CIV) = leaf inoculation, in other countries = pod inoculations. Iwaro pod test rating goes from 1 (=highly resistant) to 8 (=highly susceptible).
Witches' broom (WB) resistance reaction observed in the field over a 3-year period in Trinidad under relative low inoculum pressure (therefore among R and MR clones there may be some escape).

(4) Cotyledon weight = dry weight of beans without testa; add around 15% to obtain the dry bean weight.

(5) Pod index = number of pods for 1 kg of dry cocoa beans (vl = very low, l = low, m = medium, h = high, vh = very high).

(6) Cer = *Ceratocystis*, Mon = monilia, VSD = vascular streak dieback, SC = self-compatible, SI = self-incompatible.

Identification of off-types of clones used in the International Clone Trial using DNA analyses

O. Sounigo, A.-M. Risterucci, D. Clément, O. Fouet and C. Lanaud
CIRAD, Avenue Agropolis, 34398 Montpellier cedex 5, France

Abstract
Molecular analyses were performed in order to compare profiles of 35 clones held in the quarantine centres of Montpellier and Reading ("reference clones"), most of which were used to distribute budwood for the International Clone Trial (ICT), with clones having the same name ("test clones") that were present in collections at the sites where the ICT has been established. The main objective was to verify whether the local clones could be used as sources of budwood to establish the ICT. The analyses showed mislabelling problems for 17 out of the 35 clones tested. Differences between DNA samples collected from clones with similar names from different sites were detected in approximately 33% of the comparisons. This shows the frequent presence of off-types within and between the collections. Also a certain number of mistakes were detected in the identity of the ICT clones, apparently caused by errors in the multiplication process (e.g. PA150 and IMC47). Two ICT clones distributed were apparently different from what can be considered to be the original clones (BE10 from Belem, Brazil, and LCTEEN46 from Ecuador). Recommendations are made in order to correct identification problems in the ICT and to reduce such problems in similar future studies.

Introduction
Problems of clone misidentification have been reported when molecular profile comparisons were made between trees from the same clones within a single country (Sounigo *et al*. 2001) or between two countries (Figueira 1998). More recently, using the data available in the International Cocoa Germplasm Database (ICGD), Motilal and Butler (2003) reported differences between the same clones based on comparing their morphological descriptions in different countries. The distribution of the same set of clones to ten different countries for the International Clone Trial (ICT) established in the context of the CFC/ICCO/IPGRI project made it necessary to compare the identity of locally available clones that carry the same name as the clones that were distributed from the quarantine facilities in Montpellier (France) and in Reading (UK). Any locally available clone for the ICT could only be used in the trial if the identity of the clone is the same as that of the clone with the same name that has been distributed from the Reading and Montpellier quarantine centres. This is a prerequisite as the results of the ICT are valid only if the genotypes evaluated in different places are reliably and consistently identified.

Materials and methods
Leaf samples were collected for analyses of 35 clones mainly during the first two years of the project. When necessary, more leaf samples were taken to verify results obtained in the first two years. For each clone, the samples were collected on:

- The plants in the quarantine centres of Montpellier and Reading, which were generally used as sources of budwood by the countries which did not have the clones in their genebanks. These samples are considered as the references since they are generally the sources of budwood for most of the participating institutes.
- Trees of the genebanks (or budwood gardens) of the different participating institutes.

Between 1 and 16 different samples were collected from local genebanks for each clone and were compared to the references. According to the availability of the clone in the local collections, the samples were issued from 1 to 9 cocoa research institutes involved in the project. The number of different tree samples for each institute varied between 1 and 4.

DNA was extracted from these different samples and then submitted to polymerase chain reaction (PCR) using radio-labelled nucleotides and 10 pairs of primers designed by Lanaud *et al.* (1999) to amplify simple sequence repeat (SSR)-type sequences. The PCR products were then run on a sequencing gel before revelation using autoradiography technique.

Results

Table 1 indicates the 18 clones without mislabelling problems and the numbers of analyzed samples for each of them. Table 2 indicates the 17 clones with mislabelling problems and the number of analyzed samples with different molecular profiles observed for each of them.

Out of the 35 clones analyzed, the analyses revealed problems of mislabelling (i.e. different molecular profiles between trees ostensibly from the same clone) in the case of 33 samples out of a total of 101 individual tree samples tested. In some cases, the number of different molecular profiles for a single clone was as high as 4.

Table 3 indicates the countries with local sources of budwood that appeared to be different from the reference clones from quarantine stations.

Table 1. List of clones for which the comparison of molecular profiles did not reveal any mislabelling problem in the preliminary analyses

Clone	No. of trees analyzed	No. of countries
AMAZ15-15	4	4
APA4	2	1
EET59	4	3
GU175V	2	1
GU307V	2	1
ICS43	3	2
LAF1	2	2
LCTEEN46	2	2
Mocorongo	4	3
MXC67	1	1
N38	2	2
Playa Alta 2	6	3
RB46	2	1
SCA24	2	2
SIAL339	1	1
SIC5	1	1
SPEC54-1	2	2
TSH1188	2	2

Table 2. List of clones for which the comparison of molecular profiles revealed mislabelling problems. In the case of T60/887, no data were available for the reference. The number of profiles indicated is the total number of different molecular profiles that could be observed.

Clone	No. of trees analyzed	No. of countries	No. of molecular profiles different from the reference	No. of trees different from the reference
AMAZ5-2	1	1	1	1
BE10	1	1	1	1
EQX3360-3	3	3	1	1
GU255V	3	1	1	1
ICS1	5	4	2	2
IMC47	8	4	3	5
MAN15-2	2	2	1	1
NA33	4	4	2	3
PA107	7	4	2	3
PA120	5	3	1	1
PA150	16	7	5	3
Pound	9	5	2	1
SCA6	12	9	2	2
T60/887	5	2	4	
T79/501	10	3	2	2
T85/799	9	1	2	1
UF676	4	3	1	1

Table 3. Clones of the ICT with mislabelling problems and countries potentially concerned by these problems

Clone	Countries with genotypes different from that distributed from the quarantine stations
BE10*	Brazil
EQX3360-3	Brazil
ICS1	Cameroon, Malaysia
IMC47	Brazil, Ghana, Malaysia, Trinidad
LCTEEN46*	Ecuador
MAN15-2	Brazil
NA33	Ghana, Cameroon, Brazil
PA107	Trinidad, Brazil, Ghana
PA120	Malaysia
PA150	Trinidad, Brazil, Côte d'Ivoire, Papua New Guinea
Pound7	Trinidad
SCA6	Cameroon
UF676	Côte d'Ivoire

***** The DNA from these two reference clones appeared different from the "original" clones present in Brazil and Ecuador (the last result was obtained more recently in the laboratory of USDA, Miami)

Discussion

The results of the molecular analyses clearly indicate that if the use of local sources of budwood for the ICT could be seen as a convenient short-term solution to set up the trial, it appears to be a major potential source of problems for the proper establishment of the ICT (i.e. all genotypes should be really the same to be able to carry out genotype x environment analyses).

At present, not only the use of budwood from local sources appeared to pose a risk but also the use of several budwood sources (from more than one tree) in the quarantine centres. This was apparently the cause of off-types for the PA150 clone in PNG and Côte d'Ivoire,

with budwood received from the Montpellier quarantine centre. The results suggest that the off-types were from a second source of budwood used in Montpellier and that this plant was of a different genotype. The off-type exhibited smooth pods instead of the expected rough pods of PA150.

The results obtained here for the presence of mislabelled trees are probably underestimating the real extent of the problem. This is because usually only one tree of each budwood source was tested. One can expect that mistakes occur between trees of the same clones in many of the accessions. Also, a case was reported of two trees from supposedly the same clone found sharing the same molecular profile, while differing in the colour of their pods (Paulin, pers. comm.).

The results lead us to make recommendations for the ongoing ICT and for any future "international" or "regional" trials to be established. In both cases, the first step should consist in defining a set of primers ensuring a nearly nil probability of considering two trees as identical while they are different. The knowledge of the number of alleles and of their relative frequencies in the different cocoa populations should facilitate the choice of sets of primers resulting in the highest PIC (polymorphism information content) for each clone.

In the case of the ongoing ICT, the safest but most costly solution would involve performing molecular analyses on all the planted trees. This can seem very expensive and time-consuming but this cost should be compared to the risks of accumulating misleading data over several years which would probably result in the drawing of wrong conclusions on the estimation of "genotype x environment" interaction as well as on the value of the clones tested. A proposal is made below in order to reduce the cost of these molecular analyses without losing much in efficiency.

Three main situations can be distinguished as a basis for verifying with new DNA analyses whether any mislabelling has occurred in the establishment of the ICT:

- The trees that have provided the budwood for the trees planted in the ICT are known (generally these are the trees that were established in the budwood gardens with the ICT clones). In such a case, all these trees should have their molecular profile compared to that of the reference (Reading and Montpellier accessions). When one of them is found different from the reference, then normally all the trees planted in the trial must be compared to the reference, using only the primers which revealed the differences. The trees different from the reference should be discarded from the statistical analysis.
- The budwood-providing trees have not been identified. Then, all the potential budwood-providing trees (all the trees present on the research station that could have been used) should be compared to the reference. When one of them is found different from the reference, then all the trees planted in the trial should be compared to the reference. The trees different from the reference should be discarded from the ICT analyses.
- A third, more pragmatic, but necessarily less precise solution is to have the DNA profile made up of one of the trees planted in the ICT. If the tree sample matches the reference sample, then all other trees will be compared by visual observations, using qualitative traits such as pod shape, rugosity, flush colour, bean size, etc. If the visual observations suggest that all trees are morphologically identical, then one can be quite confident that the results of this clone can be used in the analysis of the ICT. However, if the tree is different, and all the other trees are visually similar to the same off-type, then the results of this clone cannot be used in the analyses. In the case of a mixture of off-types and the right type, only data from the right type can be used.

If, in a country, the number of right type trees representing a clone is too low, then the data obtained on this clone in this country should be discarded from the "international analysis", at least until the mislabelled trees are replaced by the right type ones (if this is possible). On the other hand, these data can be used for local analyses (in some cases the off-type can have agronomic interest). However, these off-type clones should be renamed following internationally accepted recommendations.

For establishment of future multilocation trials the following steps are proposed in order to ensure the full validity of the data obtained in such trials:

- Use of a single tree as the source of budwood for each clone, and as the source of flowers or pollen in the case of multilocation hybrid trials. Several sources of budwood or flowers can be used once these different sources have proven to share the same molecular profile;
- Perform molecular analyses on samples of plants of all the crosses or clones before planting.

The second step is less essential but would allow the detection of possible mistakes occurring in the greenhouse.

Conclusion

The results obtained from these preliminary experiments clearly show the risk of mislabelling problems in the ICT. Efficient comparative molecular analyses of the trees that have provided budwood and/or of at least some of the trees planted in the ICT appear necessary to verify if any mistakes have occurred in the source of budwood or in the multiplication process. The mislabelled trees should be discarded from the analyses of the ICT in order to avoid misinterpretation of the results. Of course, this will result in unbalanced designs and complicate the statistical analyses, resulting in the need for using software adapted to such constraints.

The future establishment of multilocation clone or hybrid trials will require a proper identification of the sources of budwood or flowers before performing the vegetative multiplication or the hand-pollinations.

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Guidelines for accelerated clone development (ACD)

Y. Efron, P. Epaina and J. Marfu

CCI, PO Box 1860, Rabaul, East New Britain, Papua New Guinea

Abstract

An accelerated clone development (ACD) scheme was designed in Papua New Guinea (PNG) to shorten the breeding cycle of clones and to address the poor correlation between the performance of individual mother progeny trees and the clones derived from them. Guidelines to the scheme as it was used in PNG are described under the headings of family selection, population size, early screening for disease resistance, direct cloning, preliminary and advanced testing and timetable. However, the scheme is flexible and can be adapted to the specific needs of any breeding programme.

Introduction

Traditionally, clones are being developed from selected individual progeny trees. However, experience has shown that there is a poor correlation between the performance of individual 'Mother' progeny trees and the clones derived from them. It is considered that the correlation can be improved by collecting field data for a longer period of time. Usually, due to the poor correlation, more mother trees are selected for cloning and the derived clones are being tested first in an observation trial. Later, selected clones are being tested in an advanced replicated trial before they are finally released to growers. Assuming an average period of eight years for testing, the breeding cycle of clones from the initial pollination to release takes about 24 years.

The concept of accelerated clone development (ACD) scheme was designed in Papua New Guinea (PNG) to address the above two major obstacles in clonal breeding – the long duration of the breeding cycle and the poor correlation between the performance of individual mother progeny trees and the derived clones. It is based on early screening for *Phytophthora* pod rot (Ppr) and vascular streak dieback (VSD), direct cloning of large number of hybrid progenies, preliminary testing of clones in an observation trial planted at high density, and advanced testing of selected clones in a replicated trial.

The following guidelines were developed based on the PNG's experience. However, the scheme is flexible. It can be modified according to the specific breeding targets, capabilities and other local considerations. It may be of particular interest and value for multi-cycled population improvement projects.

Family selection

The choice of the right families is probably the most important aspect of the scheme. In contrast to the poor correlation between individual trees and derived clones, theoretical considerations and practical experience have shown that the probability to identify desirable and high-yielding clones is greater if the better families are being used for cloning. Preliminary studies in PNG have also shown that parental clones were different in their combining abilities to provide variable proportions of high-yielding or disease-resistant clones. Unfortunately, our knowledge and experience are insufficient to enable the selection of the best possible families. Additional research is required. In the meantime, we have to rely on available knowledge, experience and a lot of intuition. It is recommended to implement the scheme with several genetically unrelated families.

Population size

Most of the economically important traits, particularly yield potential, are quantitatively inherited and show a typical normal segregating distribution. As the interest is to identify clones at the desirable tail of the curve, the higher the starting population size, the higher the probability to select high-yielding clones with the required resistance and other desirable attributes. The starting population size would depend on the number of clones that can be effectively tested in a preliminary trial. It will also depend on the availability of efficient early screening methodologies for disease resistance and the number of families to be included in the ACD scheme.

Early screening for disease resistance

An efficient screening methodology to test a large number of genotypes for resistance at an early age, preferably before cloning, is very important to the success of the ACD scheme. It enables to start with a large population size and to reduce it to a manageable size for preliminary testing with a higher proportion of resistant genotypes. Negative or positive selection can be used, depending on the nature and efficiency of the available screening methodologies. The leaf disc test for *Phytophthora* is an example of an early screening test that can be done on a large number at genotypes of the seedling stage, before cloning.

Direct cloning

Two types of buds can be used for direct cloning of individual progenies, orthotropic and plagiotropic. Orthotropic buds are available faster, directly from the nursery about 4-5 months after planting, but they tend to be dormant for a longer time as compared with plagiotropic buds. It is recommended to tip the growing point about 1-2 weeks before grafting to obtain faster and more uniform bud sprouting. If plagiotropic buds are preferred it is necessary to wait for more than a year until the jorquette is formed and the developed fan branches are sufficiently developed to provide adequate buds for grafting.

The seedlings cannot be maintained in the nursery for a long time and therefore they have to be planted in the field. A very high density of up to 20 000 seedlings/ha can be used. If primary jorquette fan branches are being used, a high proportion of the buddings, depending on the genotype, may grow orthotropically.

Propagation can be done either by grafting or by cutting. If cuttings are being used, the possible effect of the rootstock is being avoided. Grafting can be done either by patch budding or by top-grafting. In general, it is recommended to apply the standard propagation technique commonly used by the programme, or that which provides the highest success rate. If grafting is being used, it is desirable to choose rootstock as uniform as possible. Top wedge-grafting using orthotropic budwood containing one or two buds of selected seedlings is an interesting new method with potential for accelerated hybrid clone selection that may be worthwhile comparing to locally used methods. This type of top-grafting has given high success rates at a number of sites and has the advantage that bud dormancy breaks quickly.

Preliminary testing

It is desirable to include as many clones as possible in the preliminary testing. The number depends on capabilities and on the availability of land and other resources. However, considering the preliminary nature of the testing, it is possible to increase the number of clones by testing a small number of trees/clone at high density for a relatively short time. In PNG we are testing 4 trees/clone at a density of 1666 trees/ha for a period of 4-5 years from planting.

Considering the large number of clones that are being tested in the preliminary trial and the expected high variability between the clones, it is not required to follow all the clones in details. Initial selection of clones can be done based on the number of pods produced and visual observation of pods size. Yield components and measurements of other characteristics, such as resistance to *Phytophthora*, vigour, plant type, etc., are being done only in the selected clones.

The optimal time for the preliminary testing and the proportion of clones to be selected are important considerations. They are presently under investigation. However, collecting 2-3 years of yield data and selection of 5-10% of the clones for advanced testing are considered adequate.

Advanced testing

The best clones from the preliminary testing are being selected for advanced testing in a replicated trial. The advanced testing is being done according to the standard procedures used in the local breeding programme. It is recommended to pre-assess the vigour of the selected clones in order to plant them in 2-3 different sub-trials based on more uniform vigour to minimize the effect of interplant competition. Different size trees may be planted in different densities.

Timetable and activities

An example of timetable and activities as presently used in PNG is shown in Table 1 below. The estimated time required is less than 15 years, which is 10 years shorter than the traditional way. The procedures are flexible and can be modified to fit best the local conditions and the specific purpose for which the accelerated clone development scheme is being used.

Table 1. Proposed activities and timetable for the accelerated hybrid clone development scheme

Activities and time frame		No. of years
1.	Pollination to pod maturity	0.5
2.	Nursery, leaf disc test to Ppr, negative selection (25-30%)	0.3
3.1	Direct primary orthotropic budding, nursery (patch budding)	0.3
3.2	Planting in VSD “sick plot”, selection of VSD-resistant progenies, plagiotropic patch budding	1.5
4.	High density planting (1666 trees/ha), 1 replicate of 4 trees. Assess plant type, vigour, precocity, number of pods and yield components of selected clones	4-5
5.	Advanced testing of selected clones in replicated trials (4 replicates)	6-7
	Total	**12.6 – 14.6**

PHYTOPHTHORA POD ROT RESISTANCE STUDIES

Studies on the repeatability and reliability of *Phytophthora* resistance screening tests carried out in Cameroon

M.I.B. Efombagn[1], S. Nyassé[1], D. Bieysse[2], K.D. Vefonge[1] and A.B. Eskes[2]

[1] IRAD, PO Box 2067, Yaoundé, Cameroon

[2] IPGRI/CIRAD, c/o INIBAP, Parc Scientifique Agropolis II, 34397 Montpellier cedex 5, France

Abstract

Phytophthora pod rot (Ppr) disease caused by *P. megakarya* is the major constraint to cocoa production in Cameroon. The development of resistant varieties requires the use of effective resistance testing methods. The repeatability and reliability of the leaf disc and detached pod tests, as applied in selection activities in Cameroon, were studied. Repeatability of the tests was estimated by calculating the correlation (r) between resistance scores of clones, progenies and individuals within progenies in different inoculations rounds of the leaf disc and detached pod tests. Such correlations were generally significant for both tests. For the leaf disc test, as expected, correlations were lower for individuals within seedling progenies than for the average of seedling progenies or of clones. This suggests that a higher number of replicate observations are required for correct evaluation of individual seedlings than for evaluation of the average level of resistance of progenies. Observations carried out 5 or 7 days after inoculation were highly correlated, suggesting that scoring in the leaf disc test may be done only once at 5, 6 or 7 days after inoculation. In one experiment the ranking of leaf disc and detached pod inoculation test results could be compared statistically, with data being significantly correlated (r=0.78). The reliability of the tests was evaluated by correlating results of the inoculation tests with the level of field attack. These correlations were generally positive and significant, both for the leaf disc and detached pod test. A variation between mean scores of 5 and 8 in the detached pod test was related to a 35% difference in field infection with *P. megakarya* in years with medium disease pressure (29-47% average pod infection). However, correlations with field resistance were not always significant, suggesting the influence of uncontrolled environmental factors affecting field observations or the results in the screening tests. It is concluded that the leaf disc and detached pod tests, if applied under standardized conditions, can be of great value to speed up selection for Ppr-resistant varieties.

Introduction

Cocoa production in Cameroon has not increased over several decades. This stagnation is partly due to high disease and pest incidence and the lack of improved resistant and high-yielding varieties. Field performance of the planting material used by the farmers appears unsatisfactory. Traditional varieties as well as hybrids proposed by the local research stations revealed often low productivity, low vigour and high susceptibility to *Phytophthora* pod rot (Ppr). Progress obtained until the 1970s in breeding for Ppr resistance has been relatively limited.

Before 1995, significant genetic variation for Ppr attack on mature pods has been observed in cocoa breeding trials in different countries (Wood and Lass 1985; Paulin *et al*. 1994), including Cameroon (Blaha and Lotodé 1977). However, a major difficulty in selecting resistant varieties was the lack of reliable early screening tests and the lack of knowledge on the environmental versus genetic factors determining field resistance and on the stability of resistance (interaction between *Phytophthora* isolates and/or species with cocoa genotypes).

Advances in research (INGENIC 1997) have helped to overcome some of these obstacles. The target of the activities carried out at the Institute of Agricultural Research for Development (IRAD), Cameroon, within the CFC/ICCO/IPGRI project (1998-2004) was the enhancement of cocoa collections through the identification of relevant sources of resistance against Ppr and mirids among local and introduced clones, using recently developed selection approaches and methodologies. For Ppr resistance these were the tests based on leaf disc (Nyassé *et al*. 1995) and detached pod inoculation (Iwaro *et al*. 2000). The objective of this paper is to evaluate the repeatability of these resistance tests (correlation of results between inoculations series and between tests) and their reliability (predictive value in relation to the field incidence of the disease).

Methods, results and discussion

Leaf disc test

Resistance to *P. megakarya* in cocoa genotypes was assessed mainly by inoculating leaf discs taken from nursery plants (clones or seedling progenies). The screening method used was as described by Nyassé *et al.* (1995). In order to assess the repeatability and reliability of the test, correlations have been calculated based on the results of four different trials using accessions from germplasm collections and accessions collected in cocoa plantations in Cameroon. In all trials, there was a highly significant variation among the average resistance of the clones and seedling progenies and between individual plants within the progenies (where this was tested).

- **Repeatability between inoculation series**

In the first trial, four hybrid progenies introduced from Côte d'Ivoire (PA150 x T79/501, PA150 x T60/887, P7 x PA150 and P7 x T60/887) and known to be resistant to *P. palmivora* in that country were tested at IRAD. Two local clones (SNK64 and SNK413) and two "international" clones (T60/877 and T79/501), known for their relatively low susceptibility to *P. megakarya* (Nyassé *et al*. 2003), were used as controls, together with a local commercial seed garden progeny (SNK109 x T79/501) of unknown level of resistance. Eighteen leaf discs, of each of 20 seedlings for each hybrid progeny and of each of 5 budded plants for each clone were inoculated. The coefficients of rank correlation (Spearman 1904) between the three series of inoculations, carried out at different dates, and the overall means of the progenies or clones were highly significant (r=0.81-0.83). The coefficients of rank correlation between the genotype means of the individual inoculation series were slightly lower, but still significant (r=0.49-0.67). This suggests good repeatability over time for the means of the progenies or the clones. An interesting result from the trial was that the progenies from Côte d'Ivoire were in average more resistant to *P. megakarya* than the control clones and the progeny produced in Cameroon. This shows the potential of the progenies from Côte d'Ivoire for controlling Ppr in Cameroon.

In the second trial, leaf discs were used to assess average resistance to Ppr of 20 locally created seedling progenies. The rank correlation coefficients for the mean scores of the progenies for three replicates (inoculation series) were highly significant (r=0.81-0.83). Afterwards, individual seedlings of eight of the more resistant progenies were evaluated in three replications. The rank correlation coefficients for the mean scores of all individual plants in each of the three series of inoculations with the overall mean scores of the same plants were also significant (r=0.54-0.80). Between the individual series, r values were also positive and significant, varying from 0.76 to 0.88.

In the third trial, 80 farm accessions of the traditional variety planted in southern Cameroon (called "German" cocoa by the farmers) were tested. The trees were grafted

on-station and nursery leaves were assessed 9 months later for their resistance to Ppr. After three inoculation series, coefficients of rank correlation between each series and their overall means were all significant (r=0.73-0.98). However, the coefficients of correlations among the three series varied considerably (0.24-0.96), but were still statistically significant (P=0.03-0.0001).

In the fourth trial, nursery leaves of 32 introduced and locally selected clones were tested in three inoculation rounds. Rank correlation coefficients between clone means of individual series and their overall means were highly significant (r=0.75-0.90). The rank correlations among inoculation series were lower (r=0.36-0.66), though still significant (P=0.02-0.0001).

The above results indicate that genotype means in individual inoculation series were highly correlated with the genotype means of all inoculation series. However, the correlations among individual series are lower and vary more substantially. This may be ascribed to variations in factors that may affect the resistance of cocoa leaves, such as the leaf development stage and light intensity (Tahi 2003). From our results, we estimate that a minimum of two inoculation series should be carried out to assess average resistance of clones or of seedling progenies, and at least three inoculation series need to be done to estimate resistance of individual seedlings. However, if the correlations among series are low, more inoculation series will need to be carried out to obtain a reliable estimate of the resistance.

- **Correlations between observations carried out at 5 and 7 days after inoculation**

In trials 1 and 2 (see above), coefficients of rank correlation for observations made 5 and 7 days after inoculation were identical and highly significant (r=0.91). The coefficients of correlation between scores at 5 and 7 days were also highly significant, varying from 0.58 to 0.98 in trial 3 and from 0.88 to 0.98 in trial 4. These results suggest that in the leaf disc test it is sufficient to carry out scoring of Ppr symptoms only once, either at 5, 6 or 7 days after inoculation. Scoring should preferably be done on either of these dates, when the intensity of symptoms allows good separation of susceptible and resistance control genotypes.

- **Comparison of the leaf disc test with field results**

Earlier studies in Cameroon have shown good correlation between leaf disc test results and field results for six parental clones of a diallel mating design planted in Barombi-Kang in 1974 (Nyassé *et al.* 2002). Correlations between the leaf disc test and field data could be calculated for trial 1 and trial 4.

In trial 1, the comparison of the leaf disc test results for two international control clones (T79/501 and T60/877) and for the four progenies introduced from Côte d'Ivoire showed a good correlation with field results obtained with the clones involved as parents in the four crosses in Côte d'Ivoire (P7, PA150, T79/501 and T60/877). The highest resistance in the leaf disc test was observed for the cross between the two clones (P7 and PA150) with highest field resistance in Côte d'Ivoire (Nyassé *et al*. 2003). These comparisons between Côte d'Ivoire and Cameroon suggest reliability of the leaf disc test, even if the *Phytophthora* species present in the two countries are different.

Leaf disc test results obtained in trial 4, using nursery leaves from 12 clones of the SNK600 series (cloned F1 hybrids locally selected for resistance to Ppr), were compared to field infection in 1996 and 1997 of the same clones (Table 1) observed through weekly counting of Ppr-infected cherelles and pods. It should be noted that the field conditions (tree size, overhead shade, number of trees per clone) were highly variable and the number of trees sometimes low in the Nkoemvone collection where the observations were made. This may be the reason why the results of 1996 were not significantly correlated with the results of 1997 (Table 1). The rank correlation between the leaf disc results and the 1997 field data were

significant with r=0.86 (P=0.02), but not so for the 1996 field data (r=0.17). This result suggests that the 1996 field data may possibly be less reliable than the 1997 field data.

Table 1. Percentage of field infection level of 13 SNK clones observed in 1996 and 1997, and mean disease scores obtained with the detached pod test and with the leaf disc test (3 replicates)

Clone	Field Ppr (%)		Leaf disc test	Pod test
	1996	1997		
SNK615	22	6	2.81	3.33
SNK614	18	2	2.00	3.83
SNK619	9	9	2.59	4.00
SNK613	50	5	2.73	4.75
SNK608	7	10	3.88	4.83
SNK630	49	5	2.31	4.83
SNK620	7	2	2.77	5.00
SNK622	14	12	3.00	5.00
SNK625	8	1	2.50	5.50
SNK602	4	17	3.19	5.58
SNK624	20	15	2.73	5.91
SNK600	15	21	3.09	6.00
SNK607	15	15	3.69	6.75

Detached pod test

Two trials were carried out during the project to evaluate resistance according to the method developed by Iwaro *et al.* (2000), inoculating 10 pods per replicate and scoring disease severity at 4 days after inoculation. In the first trial, the objective was to evaluate 16 clones of the SNK600 series and to compare results with field observations. The SNK clones had been selected as they had shown relatively low infection levels in the field (Nkoemvone collection). In the second trial, detached pod inoculations were carried out on four clones, with well-known levels of field resistance, used as parents for a diallel crossing design (Nyassé *et al.* 2002) and two other clones (ICS1 and T79/501).

- **Repeatability of test results**

Table 2 presents the results obtained with the detached pod test after three inoculation series (replicates) of 16 SNK clones. Average disease scores of these clones varied from 3.3 to 6.8, therefore possibly very resistant (scores 1 and 2) and very susceptible (scores 7 and 8) genotypes were not represented among the clones tested. Correlations were positive and significant between each of the three replicates and their overall mean (r=0.43-0.63, and P=0.02-0.008). The means of the clones for each of the three inoculation series were also positively correlated to each other, with r=0.55-0.65 (P=0.02-0001).

Table 3 presents the mean disease score of six clones after three replicates of pod inoculations. Levels of resistance of these clones as identified in earlier studies with inoculation of attached wounded pods (Nyassé *et al.* 1995) and leaf disc inoculations (Nyassé *et al.* 2002) were confirmed with the detached pod test used in the present study, confirming good relationship between tests. With the detached pod test (Table 3), the replicate means were positively correlated to the overall means with r varying from 0.87 to 0.90 (P=0.04-0.02). Correlations between replicates were also positive, varying from 0.60 to 0.89 (P=0.04-0.01).

Table 2. Evaluation with detached pod inoculations and leaf disc test of 16 SNK clones selected for field resistance to Ppr

SNK clone number	Replicate 1 (10 pods)	Replicate 2 (10 pods)	Replicate 3 (10 pods)	Means (pod test)	Means (leaf disc test)
615	2.2	3.5	4.3	3.3	2.81
614	5.8	1.5	4.3	3.8	2.00
619	4.8	4.0	3.3	4.0	2.59
611	3.3	4.5	5.3	4.3	2.90
613	4.8	4.5	5.0	4.8	2.73
603	3.8	4.8	5.8	4.8	2.81
630	4.3	5.0	5.3	4.8	2.31
608	4.3	4.0	6.3	4.8	3.88
620	4.0	5.5	5.5	5.0	2.77
622	5.0	4.0	6.0	5.0	3.00
625	6.8	4.0	5.8	5.5	2.50
602	5.0	5.8	6.0	5.6	3.19
624	6.5	5.5	5.8	5.9	2.73
600	6.0	5.8	6.3	6.0	3.09
633	6.0	6.3	6.3	6.2	3.03
607	6.8	6.8	6.8	6.8	3.69

Table 3. Resistance of parental clones of a diallel mating design established in the Kumba/Barombi-Kang station of IRAD assessed with the detached pod test

Clone	Replicate 1	Replicate 2	Replicate 3	Mean
UPA134	5.3	4.9	4.5	4.9
ICS1	4.3	4.5	6.0	4.9
T79/501	5.8	5.2	4.8	5.3
SNK413	5.5	6.2	6.0	5.9
ICS84	6.8	6.0	5.7	6.2
SNK10	7.8	8.0	8.0	7.9

- **Correlation of the detached pod test and field results**

Comparisons from two sets of data could be made to verify the relationship between the detached pot test and the incidence of the Ppr disease in the field. Firstly, the field infection level of 13 SNK clones, observed for two years (1996 and 1997) at the IRAD station in Nkoemvone, was compared with results of the detached pod test applied to the same clones (see Table 2). Correlation between the field data for 1997 and the detached pod test results (Table 1) was positive (r=0.59) and significant (P=0.03). In contrast, a negative correlation (r=0.16) was obtained between the field data for 1996 and the detached pod test results. This result was similar to that obtained with the leaf disc test (see above). This may therefore suggest again that the 1996 field data were less reliable than those of 1997.

Secondly, field Ppr incidence of four parental clones from the diallel mating design observed in 1999, 2000 and 2001 in a completely randomized clone trial with 20 trees per clone (Efombagn *et al*. 2004). High rainfall had induced very high infection levels in 2001 (Table 4). The field results were compared to the average results of the detached pod test applied to the same clones (Table 4). Good correlation between field incidences over the three years was observed. Coefficients of rank correlations and their probabilities are presented in Table 5. They show a high level of reliability of the detached pod test.

Table 4. Percentage of field Ppr infection, observed for 3 years, and mean disease scores from the detached pod test obtained with four parental clones of a diallel mating design

Clone	Field Ppr (%)			Pod test (mean)
	1999	2000	2001	
SNK10	52.7	69.1	93.4	7.9
ICS84	26.3	53.5	80.2	6.2
SNK413	25.1	45.4	79.6	5.9
UPA134	13.2	19.5	79.7	4.9
Mean	**29.3**	**46.9**	**83.2**	**6.2**

Table 5. Spearman's rank correlation of four parental clones of the diallel mating design, based on percentage of rotten pods for 3 years (1999, 2000 and 2001) and mean disease scores in the detached pod test

Methods compared		Field Ppr (%)		Pod test (mean scores)
		2000	2001	
Field Ppr (%)	1999	0.91* (0.08)**	0.95 (0.04)	0.99 (0.007)
	2000	-	0.76 (0.23)	0.95 (0.04)
	2001		-	0.92 (0.07)

* Coefficient of rank correlation

** Probability

Correlations between leaf disc and detached pod tests

Correlation between the leaf disc and the detached pod test results could be analyzed in the trials presented above, which included locally selected and introduced clones. Mean pod test scores of all the 16 clones of the SNK600 series (Table 2) were significantly correlated with mean scores in the leaf disc test ($r=0.78$, $P=0.02$). A comparison is made for four of the six parental clones used in the diallel mating design that were screened in the project with the leaf disc and pod inoculation tests (Table 3). All clones were similarly ranked in both tests. Earlier studies (Nyassé *et al*. 1995; Nyassé 1997) had already shown similar ranking between leaf disc and attached pod test results for all six parental clones (SNK10, SNK413, ICS84, ICS95, IMC67 and UPA134).

General discussion and conclusions

The studies carried out have shown that the leaf disc and detached pod tests for evaluation of Ppr resistance can be both repeatable and reliable. The level of repeatability of the test results influence the number of replicates that have to be carried out in order to obtain reliable results.

In our studies, progeny or clone means were significantly correlated between replicates for both test methods.

From our experience, in the leaf disc test two replicates are considered enough to estimate the average susceptibility of clones or seedling progenies when applying the standard method (Blaha *et al.* 2000), with inoculation of 40-60 discs from 3 to 5 clonal plants or from 10 to 20 seedling plants per progeny. However, for evaluation of individual plants within progenies with the leaf disc test, the correlations were low and less significant. This can be ascribed to the relatively low number of leaf discs (18) tested for each individual seedling in each replicate, and also to the fact that leaves of individual plants are not always at the right development stage. Therefore we estimate that at least three replicates are required for evaluation of individual seedlings, with 18 leaf discs per replicate. When the correlations

between inoculation series are low, this would mean that some of the factors affecting the reactions of the tissue in the tests, such as leaf development stage and light intensity (Tahi 2003), have not been sufficiently uniform. In such cases, inoculations will need to be repeated more times, both for evaluation of average resistance of progenies or clones and for that of individual seedlings, until correlations between replicates become significant.

In the leaf disc test, we have sometimes observed that the result of one replicate does not correlate well with the overall means nor with the other replicates. It may then be wise to eliminate the results of such a replicate from the analyses. This has happened in trial 1 (see above, under leaf disc test results). Leaves of the local control progeny SNK109 x T79/501 were obtained in the first inoculation round from a different, more shaded nursery than the leaves of the other genotypes. The results of the first inoculation round showed higher resistance of this progeny than that of the resistant progenies introduced from Côte d'Ivoire. The seedlings of the SNK109 x T79/501 progeny were then placed in the same nursery as all other varieties. The following inoculation series, carried out 2 months later, showed that the introduced progenies were much more resistant than the local progeny SNK109 x T79/501. The results of the first inoculation round were discarded because the relatively higher resistance of the SNK109 x T79/501 progeny was likely induced by the higher shade levels in the nursery where it was growing when the first replicate was carried out.

For the detached pod test, according to our experience, three replicates of 10 pods each are required for accurate estimation of resistance. However, many of our clones had intermediate resistance responses (Table 1). Therefore it is possible that two replicates may be sufficient for screening purposes. More results are needed to decide on the exact number of replicates.

Highly significant effects of cocoa genotypes (hybrid progenies, seedlings within progenies and clones) for resistance to *P. megakarya* were detected with the leaf disc and detached pod test. Such large genetic variation agrees with the results of other authors, e.g. Blaha and Lotodé (1977), Nyassé *et al.* (1994) and Iwaro *et al.* (1997), and can apparently be correlated with field results (this paper; Tahi *et al*. 2000; Iwaro *et al*. 2005). For example, a variation of 5 to 8 on the 8-point assessment scale used in the detached pod test may mean a difference of 35% infection in the field with *P. megakarya* (Table 4) in years with average disease incidence of 30 to 45% (1999 and 2000). Tahi *et al.* (2000) have shown that 2 points on the 0-to-5-point assessment scale for the leaf disc test may mean a 20% difference of field infection in the case of *P. palmivora* in Côte d'Ivoire, with average infection level of 15%. Therefore, efficient and rapid selection of cocoa genotypes with the leaf disc and detached pod tests seem feasible, at least when the conditions under which these tests are carried out are sufficiently standardized.

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Efficiency of methods to evaluate cocoa resistance to *Phytophthora* pod rot in studies carried out in Côte d'Ivoire

I.B. Kébé[1], G.M. Tahi[1] and A.B. Eskes[2]

[1] CNRA, 01 BP 1740 Abidjan 01, Côte d'Ivoire

[2] CIRAD-CP, TA 80/02, 34398, Montpellier, France

Abstract

The objective of this paper is to analyze the repeatability and reliability of two screening methods recommended in the CFC/ICCO/IPGRI project to evaluate resistance to *Phytophthora* pod rot (Ppr). The results presented here correspond to the application of the leaf disc and the detached pod tests (carried out without wounding) on cocoa genotypes with known levels of field resistance to Ppr.

Broad-sense heritability for the leaf disc test varied between individual inoculation series from 0.17 to 0.58, and average heritability increased from 0.37 to 0.64 with one to four replications of the test carried out over time. Significant correlations were obtained in several trials between disease scores observed in the leaf disc test and natural level of Ppr infection of cocoa seedling progenies as well as for clones in the field (r=0.74-0.95). Correlations were higher when field data were based on weekly observations on the percentage of rotten pods and cherelles carried out during the epidemic period than on the percentage of rotten pods counted monthly at harvesting time over the entire year. Similar levels of significant positive linear correlations (r=0.84 to 0.95) were also observed for the detached pod tests and field results. The linear correlation between the detached pod and leaf disc test was also highly significant (r=0.86).

The repeatability for the detached pod test was very good, with coefficients of rank correlation varying from 0.77 to 0.94. One replicate with five pods can therefore already provide reliable results. The repeatability for the leaf disc test was high for averages of seedling or adult tree progenies, with coefficients of rank correlations between series varying from 0.74 to 0.90. For individual seedlings in the nursery and individual field trees, the coefficients of rank correlations between inoculation series were lower (0.36-0.63 and 0.20-0.49, respectively), but still highly significant. With such levels of repeatability, it is recommended to use two inoculation series for the evaluation of the average resistance of progenies or clones, if these are represented by many plants. For selection of individual plants, at least three replicates should be used for nursery seedlings and four for individual field plants.

The results suggest that routine application of the leaf disc test and of the detached pod test, if carried out under uniform conditions and in a standardized manner, should both be efficient in selection of new varieties with high levels of field resistance. The leaf disc test is recommended for early resistance screening, whereas the detached pod test is recommended for screening of resistance of adult trees.

Introduction

Phytophthora pod rot (Ppr) is the major disease of cocoa in Côte d'Ivoire. The losses increased after *P. megakarya* was detected in the eastern part of the country by the end of the 1990s (Koné and Kébé 1999). In areas invaded by *P. megakarya*, the pod losses have increased from an average of 15% in the presence of *P. palmivora* to 30-35% in the presence of *P. megakarya* (Belin and Bonaventure 1961; Kébé 1999). Selection for resistant cocoa varieties has become a priority for the Centre National de Recherches Agronomiques (CNRA) and considerable

efforts were undertaken to develop and/or improve resistance evaluation methods. The efficiency and reliability of these methods will condition the speed with which new resistant varieties can be developed. The present paper aims at testing the validity and reliability of resistance evaluation methods applied in the CFC/ICCO/IPGRI project on "*Germplasm Utilization and Conservation: a Global Approach*".

The research on resistance methods was carried out in three phases:

- From 1994 to 1996, early screening methods using inoculation of leaves, twigs and roots were tested and significant correlations with field results established with all tests. The leaf test was chosen as the most promising method for early resistance screening (Tahi *et al*. 2000).
- Between 1997 and 1998, the leaf disc test was standardized and its repeatability evaluated. In addition, heritability estimates were calculated (Tahi *et al*. 2006)
- From 1998 to 2003, the leaf disc test was applied on a large scale in the breeding programme to evaluate clones and crosses that have been planted in field trials.
- In 2002 and in 2005, detached pod inoculation tests were carried out on genotypes with known level of resistance according to the leaf disc test and field results.

Materials and methods

Plant materials used for methodological experiments

To compare resistance tests and field results, inoculations were carried out on entire leaves, leaf discs and pods of 18 different clones. These were chosen because of their known level of field resistance to Ppr (Table 1). The level of field infection (percentage of Ppr) was based on 6 years' field observations on nine clones in trial BL7, that was planted in 1986 in Bingerville, Côte d'Ivoire (Anonymous 1998), and on the general combining ability (GCA) values calculated for Ppr infection of nine parental clones based on 10 years' observations in trial B8, that was planted in 1979 also in Bingerville (Cilas *et al*. 1998; Tahi *et al*. 2000). The IFC5 clone, growing in the Bingerville collection, was used as a susceptible control clone. All clones were growing without any overhead shade.

To estimate the repeatability and heritability of the leaf disc test, the following crossing designs were used:

- A 5 x 5 partial diallel mating design involving 10 crosses and 5 selfings, each with 30 nursery seedlings of 5-8 months old. The parents were known for their yield potential and for their variable levels of susceptibility to *P. palmivora*. These were: PA150 (resistant), P7 (resistant), T60/887 (moderately resistant), "H" (moderately resistant tree selected from the cross UPA402 x UF676) and IFC1 (susceptible). The progenies were deployed in three blocks in the nursery, each containing 10 plants per progeny. The parental clones, each represented by five rooted cuttings, were placed nearby the seedling progenies.
- A 4 x 2 factorial mating design, which is part of a larger field trial (C2/1) planted at the research station of CNRA in Bingerville, Côte d'Ivoire. Each of the 8 progenies comprised 30 trees that were 11-year-old at the time of the leaf disc inoculations. The field design was composed of totally randomized single trees, planted in three adjacent blocks at a density of 1333 trees/ha. The female parental clones (PA13, P19A, PA121 and IS89) and male parental clones (IMC67 and PA150) were each represented by five adult trees planted in rows in a small germplasm collection, which was located nearby the factorial progeny trial. Three trees per parent were used for the leaf disc inoculation tests.

Table 1. Level of field resistance to *Phytophthora* pod rot (Ppr) and genetic origin of clones studied (UA = Upper Amazon origin)

Field trial	Clone number	Genetic origin	Field Ppr (%)		
Bingerville (clone trial BL7)	332	P7 x IFC5 (UA x Amelonado)	5.6	a*	
	735	PA150 x IFC1 (UA x Amelonado)	10.0	ab	
	1041	SCA6 x IFC5 (UA x Amelonado)	11.5	abc	
	1020	T85/799 x IFC15 (UA x Amelonado)	13.7	bc	
	1514	T60/887 x IFC5 (UA x Amelonado)	16.3	de	
	330	IMC67 x IFC1 (UA x Amelonado)	16.5	de	
	1202	NA79 x IFC1 (UA x Amelonado)	17.2	e	
	202	T60/887 x IFC1 (UA x Amelonado)	23.4	f	
	1633	T63/967 x IFC5 (UA x Amelonado)	25.7	f	
Bingerville (parental clones of hybrid trial B8 planted in B10)	SCA6	UA	11.5**	a	5.5***
	P7	UA	13.7	a	6.8
	PA150	UA	11.1	a	9.7
	T60/887	PA7 x NA32 (UA x UA)	19.3	bc	10.8
	T85/799	IMC60 x NA32 (UA x UA)	18.8	b	18.7
	IMC67	UA	25.1	bcd	16.7
	IMC78	UA	28.7	d	19.9
	NA32	UA	24.5	bcd	23.0
	NA79	UA	26.7	cd	24.5
Control clone	IFC5	Amelonado			

* Different letters indicate significant differences within columns or rows (for means) according to the Newman and Keuls test at 5 % probability (Anonymous 1998)

** General combining ability expressed as average % of rotten mature pods in trial B8 based on 10 years' field data (Cilas *et al*. 1998)

*** General combining ability for the % of rotten cherelles and mature pods observed on progenies in the B8 trial in 1990, by weekly field observations

Application of the leaf disc test in project trials

During the project, the following materials were tested by standardized application of the leaf disc test (Nyassé *et al*. 1995), according to the method as described by Blaha *et al*. (2000), and by using two or three replicates in time (inoculation series 1, 2 and 3):

- 37 parental clones of the recurrent selection programme,
- 149 hybrid progenies,
- 23 genotypes of the Local Clone Trial, and
- 25 genotypes of the International Clone Trial.

Studies using pod inoculations

Detached pod inoculations without wounding, as proposed by Iwaro (2000), were carried out on eight parental clones of B8 and eight clones of BL7 (Table 1). Details on the methods used are provided together with the results (see below).

Results and discussion

Inoculation of leaves or leaf discs

- **Correlation between inoculations of leaves or leaf discs and field resistance**

The coefficients of correlation between average percentage of field Ppr infection for hybrids or clones and the disease scores (DS) obtained with entire leaf or leaf disc inoculations were positive (r=0.74-0.95) and highly significant in most cases (Table 2). For individual tree data, the coefficient of correlation of repeated leaf disc inoculations and field results was lower (r=0.32), but still significant. This can be ascribed to lower precision (and heritability) for both field resistance and leaf disc resistance, when individual plants are evaluated instead of crosses or clones represented each by many plants.

For the evaluations of the parental clones of B8 growing in B10 and their progenies growing in B8, it appeared that the leaf or leaf disc inoculation test results were better correlated with field results when the latter were obtained through weekly counting and elimination of Ppr infected cherelles and pods during the 1990 epidemic, as compared to 10 years' monthly observations on Ppr infection at harvesting. This suggests that weekly observations on infected cherelles and pods during an epidemic cycle may provide a more reliable estimate of the genetic resistance to infection than the monthly harvesting data over many years, which involved only mature and ripe pods. It may be that the latter data, besides resistance to infection, include also other mechanisms of field resistance, such as escapes due to out-of-season bearing of ripe pods.

Table 2. Coefficients of rank correlation between field level of attack by *P. palmivora* and inoculation tests carried out on leaves and leaf discs

Genotypes (material inoculated)	No. of genotypes	Field data x leaf test			
		No. of years of field data			
		1	6	8	10
Clones of B10 (discs from field leaves)	9	0.91**	-	-	0.81*
Clones of BL7 (discs from field leaves)	9	-	0.86**	-	-
Hybrids in B8 (nursery leaves)	9	0.95**	-	-	0.88**
Hybrids in C2/1 (discs from field leaves)	8	-	-	0.74**	-
Individual trees in C2/1 (discs from field leaves)	232	-	-	0.32**	-

* significant at 5%

** significant at 1%

- **Repeatability of leaf disc test results**

The 6- to 8-month-old seedlings of the diallel crossing design were inoculated four times using leaves collected from nursery plants. For the 4 x 2 factorial trial, leaf disc tests were also carried out four times on 30 trees per cross. The coefficients of rank correlation for the average DS values per plant (seedling or adult trees) and per cross for each inoculation series (S1 to S4) with the mean DS values obtained in all four leaf disc inoculation series were similar for leaves taken from nursery materials (Table 3) and for leaves taken from field plants (Table 4).

Rank correlations between inoculation series were always highly significant, but, as expected, lower for individual plants (r=0.22-0.63) than for the average DS values of the crosses (r=0.74-0.90) in the diallel and factorial designs (Tables 3 and 4). Correlations between individual series and the average of four series were relatively high (r=0.67-0.82) for individual plants and very high (r=0.88-0.96) for the averages of progeny means.

This suggests that two series would be enough to obtain reliable average scores in the leaf disc tests for progeny means, whereas for individual plant observations (in the nursery or in the field) three to four inoculation series may be required.

Table 3. Coefficients of rank correlation between average disease scores (DS) obtained in four inoculation series (S1 to S4) for 15 crosses in a 5 x 5 partial diallel crossing design, using leaf discs obtained from nursery leaves. Average coefficients for progeny means are given in italic (above the diagonal) and for individual nursery seedlings in normal font (below the diagonal).

Serie	S1	S2	S3	S4	Mean DS (S1 to S4)
S1		*0.78****	*0.87****	*0.89****	*0.96****
S2	0.36***		*0.74****	*0.76****	*0.88****
S3	0.42***	0.41***		*0.87****	*0.93****
S4	0.44***	0.44***	0.63***		*0.94****
Mean DS (S1 to S4)	0.72***	0.72***	0.82***	0.81***	

*** All coefficients of correlation were significant at 0.1% probability

Table 4. Coefficients of rank correlation between average diseases scores (DS) obtained in four inoculation series (S1 to S4) for 8 crosses of a 4 x 2 crossing design, using leaf discs obtained from adult trees grown in the field. Average coefficients of correlation for crosses are indicated in italic (above the diagonal) and for individual trees in normal font (below the diagonal).

Serie	S1	S2	S3	S4	Mean DS (S1 to S4)
S1		*0.87****	*0.90****	*0.82****	*0.94****
S2	044***		*0.86****	*0.87****	*0.95****
S3	0.20***	0.26***		*0.81****	*0.95****
S4	0.22***	0.36***	0.49***		*0.93****
Mean DS (S1 to S4)	0.71***	0.72***	0.67***	0.72***	

*** All coefficients of correlation were significant at 0.1% probability

- **Heritability estimates obtained with the leaf disc test**

Narrow- and broad-sense heritabilities were calculated for the mean disease scores obtained in the four inoculation leaf disc series (replicates) for the diallel and factorial cross designs, containing 10 and 8 crosses respectively (Table 5). These heritabilities were based on individual plant data, i.e. for 300 seedling plants in the nursery for the diallel and 240 adult trees in the factorial crossing design.

For individual replicates, the heritabilities for DS values in the leaf disc tests varied considerably (Tahi *et al*. 2006), which suggests that the heritabilities vary according to the test conditions. The mean heritability values increased with the number of replicates (Table 5). However, there was no substantial increase beyond three inoculation series (replicates), which would therefore be sufficient to select efficiently for resistance to Ppr with the leaf disc tests.

Narrow-sense heritabilities were lower than broad-sense heritabilities for the diallel crossing design, suggesting dominant as well as additive gene action. Similar narrow- and broad-sense heritabilities for the factorial design suggest mainly additive gene actions in this design.

Table 5. Average individual plant heritabilities for disease scores observed in leaf disc tests estimated in a partial diallel and in a factorial crossing design with increasing number of inoculation series (replicates)

Heritabilities (h^2)		Nursery plants (diallel) No. of replicates				Field trees (factorial) No. of replicates			
		1	2	3	4	1	2	3	4
Means	**Narrow-sense**	0.23	0.29	0.32	0.34	0.36	0.51	0.61	0.67
	Broad-sense	0.39	0.51	0.57	0.60	0.37	0.53	0.62	0.67

Broad-sense individual plant heritabilities were similar for nursery plants (diallel) and adult field plants (factorial). The relative high heritabilities obtained with three replicates (h^2 of about 0.6) and the considerable variation in the level of attack in the field for individual trees in the factorial design (2 to 31 Ppr infection), suggest that individual plant selection might be very effective by using the leaf disc test.

- **Repeatability observed in routine leaf disc inoculation tests applied in project trials**

Four large early Ppr resistance screening experiments were carried out in the course of the project. The relative high coefficients of rank correlation (r=0.55-0.90) between average DS values between inoculation series show that good repeatability of the test results can be obtained with different types of test materials (Table 6).

Table 6. Coefficients of correlation between mean DS values observed for clones or hybrid families in leaf disc inoculation series in routine Ppr resistance screening experiments

Breeding materials tested	Coefficient of correlation between mean DS scores in inoculation series (S)		
	S1/S2	S1/S3	S2/S3
Parents of recurrent selection programme	0.78***	0.63***	0.66***
Local clones	0.82***	0.73***	0.90***
Inter-group hybrids	0.79***		
International clones	0.55*		

*** = significant at 0.1% probability
* = significant at 5% probability

Tests with detached pod inoculations

- **Correlation between inoculations of detached pods and field resistance**

For the inoculations only 4-month-old pods obtained by hand-pollinations were used. Only coefficients of linear correlation with field results are presented here.

In the first trial, one series of inoculations was carried out in 2002 and two series in 2005. In 2002, five detached pods each of eight parental clones of the B8 hybrid trial were inoculated in one replicate (Table 7). Disease scores observed 4 days after inoculation varied from 1 to 7.2 and the differences between clones were highly significant. The coefficient of variation was 21.5%. The coefficient of linear correlation (Pearson) with the field results (weekly observations carried out in 1990) was highly significant (r=0.92). In 2005, the two replicates were carried out at 3-day interval between inoculation dates. In each replicate four pods from three different trees were inoculated per clone and scoring was done at 4 and 6 days after inoculation. The statistical analyses for the means of the two replicates with scoring at 4 and 6 days showed highly significant clone effects (Table 7), with very similar scores to the ones obtained in the 2002 replicate. For the clone averages, the linear correlations between the results and the field data were again very significant (r=0.95 and 0.93 for scoring at 4 and 6 days, respectively). As expected, scores at 4 and 6 days were also significantly correlated (r=0.98) and the effect of the trees within clones was not significant in this trial.

Table 7. Comparison of resistance of detached pods inoculated by spraying and field level of attack of eight cocoa clones growing in the B10 collection

Trial	Parental clones	Test 1 *	Test 2 **		Field level of attack (%Ppr)***
		Scoring at 4 days	Scoring at 4 days	Scoring at 6 days	
Parental clones of hybrid trial B8 (growing in the B10 collection)	SCA6	1.0 a	1.4 a	1.6 a	5.5
	P7	1.2 a	1.3 a	1.3 a	6.8
	PA150	1.8 a	1.6 a	1.8 a	9.7
	T60/887	3.4 b	4.1 b	5.0 b	10.8
	T85/799	7.0 c	4.6 c	6.0 c	18.7
	IMC67	7.2 c	5.3 d	6.4 cd	16.7
	NA32	7.2 c	7.3 f	7.8 f	23.0
	NA79	6.4 c	6.8 f	7.4 e	24.5
Control	IFC5	6.8 c	6.3 e	6.9 de	
Mean		4.7	4.3	4.9	
CV		21.5%	20.5%	16.9%	

* Test 1 was carried out in 2002 with only one replicate of five pods inoculated and observed at 4 days after inoculation

** Test 2 was repeated twice in 2005, using four pods of each of three trees per clone in each inoculation series, with scoring at 4 and 6 days after inoculation

*** GCA values for the % of rotten cherelles and mature pods observed on progenies in the B8 trial in 1990, by weekly field observations

In the second trial, nine genotypes of the BL7 clone trial were used. One series of inoculation was carried out in 2005, by inoculating four pods of each of three trees of each clone. A resistant (P7) and a susceptible (NA32) clone from the B10 collection were used as controls (see Table 7). Results showed highly significant clone effects both for scoring at 4 and 6 days after inoculation (Table 8). Linear correlations with field results were highly significant both for 4 (r=0.91) and 6 days (r=0.84). Scores at 4 and 6 days were also significantly correlated (r=0.95) and the tree effect was again not significant.

- **Repeatability of the detached pod inoculation results**

In the first trial, three inoculation series were carried out, one in 2002 and two in 2005. The coefficients of linear correlation of the mean scores of series 1 with those of series 2 and 3 were highly significant (0.94 and 0.91, respectively), as was the correlation between series 2 and 3 (r = 0.98). The coefficients of rank correlation varied between 0.77 and 0.99.

Table 8. Comparison of resistance of detached pods inoculated by spraying and field level of attack of eight cocoa clones growing in the BL7 clone trial

Clone number	Origin of clones	Test 2005 *		Field level of attack **
		Scoring at 4 days	Scoring at 6 days	
Control	P7	1.0 a	1.0 a	-
332	P7 x IFC5	1.5 a	1.5 a	5.6
1020	T85/799 x IFC15	3.3 b	4.7 b	13.7
240	T85/799 x IFC1	4.3 b	5.3 b	13.6
1938	PA7 x IFC15	4.8 cd	5.3 b	18.7
330	IMC67 x IFC1	5.2 cd	6.4 c	16.5
537	T79/416 x IFC5	5.2 cd	6.8 c	12.4
1514	T60/887 x IFC5	5.7 d	6.3 c	16.3
Control	NA32	6.8 e	7.7 d	-
1633	PA7 x IFC15	8.0 f	8.0 d	25.7
Mean		4.6	5.3	
CV		20.6%	19.4%	

***** The test was carried out in 2005, using four pods of each of three trees per clone, with scoring at 4 and 6 days after inoculation

** Percentage of rotten mature pods observed at harvesting time of clones in the BL7 trial over 5 years (1989-1994)

Correlation between the leaf disc and pod inoculation tests

Only for the first trial set-up of the detached pod inoculations, a comparison could be made between the average scores in the three series of pod inoculations with the results of the leaf inoculation test on the same clones (Tahi *et al*. 2000). The coefficient of rank correlation was significant at 1% probability (r=0.86) and the coefficient of linear correlation at 5% probability (r=0.77).

General discussion and conclusions

The results obtained show the reliability and good repeatability of the leaf disc and detached pod inoculation test. The significant correlations with field results confirm earlier work published by Tahi *et al*. (2000) for the leaf disc test and Iwaro *et al*. (2005) for the detached pod test. These tests, when used in a standardized manner, can therefore be recommended for routine screening aiming at selection of cocoa varieties with high levels of resistance to Ppr. The leaf disc test is recommended for early screening of nursery plants, whereas the detached pod test is recommended for screening of resistance of adult trees.

For the leaf disc test, our results suggest that two replicates (series of inoculations carried out with a time interval of some weeks) could be enough to evaluate in a reliable manner the average level of resistance of seedling progenies or clones, if these are represented by a large number of plants. For correct estimation of the resistance of individual plants (nursery seedlings or adult field trees) at least three replicates are required. If the correlations between the average disease scores between inoculation series are low, it could be that some of the factors that affect the results could not be controlled sufficiently. In this case, the test needs to be repeated more times, until the correlations between replicates become significant. Special attention needs to be paid to the availability of leaves at the right development stage for all treatments and to the uniformity of the exposure of the leaves to light, as these factors may significantly modify the results (Tahi 2003).

For the detached pod test, our results suggest that one replicate with five pods can already provide reliable information. This is similar to the number of 3 pods with 2 replicates, as recommended by Iwaro (2000). However, in our tests the age of the pods (4 months old) was controlled through manual pollinations. It might be that, if the recommended age of the pods chosen for the test needs to be inferred from the size of the pods, the reactions to infection may be more variable. In that case, two replicates of five pods each may be required. Scoring of symptoms at 4 and 6 days after inoculation were very well correlated, however scoring at 4 days resulted in a slightly better correlation with field results in the second trial.

It is also important to note that the results of the pod and leaf disc tests were well correlated. This was somehow expected, as both tests are correlated with field results.

It is possible that the good results obtained in our studies have been favoured by the relative large genetic variation for resistance of the materials that were used in the tests. Indeed, the genotypes tested were chosen because of their known variation in response to Ppr infection. When materials are tested with low genetic variation for resistance, it will be more difficult to find such significant correlations among test and between tests and field results.

Acknowledgements

The authors are grateful for the financial support received through the CFC/ICCO/IPGRI project. We also wish to thank the research assistant, Mr Bakary Coulibaly, and the laboratory staff of the CNRA, Mr Bernard Abe, Mr Ouglan Traore and Mr Laurent Toure, for their dedicated involvement in the execution of the tests.

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Validation studies on the detached pod test and leaf disc inoculation method for the assessment of cocoa resistance to *Phytophthora* infection

A.D. Iwaro[1], J.-M. Thévenin[2], D.R. Butler[1] and A.B. Eskes[3]

[1] Cocoa Research Unit (CRU), The University of the West Indies, St. Augustine, Trinidad and Tobago
[2] CIRAD, Avenue Agropolis, TA80/02, 34398 Montpellier cedex 5, France
[3] IPGRI/CIRAD, c/o INIBAP, Parc Scientifique Agropolis II, 34397 Montpellier cedex 5, France

Abstract

During the 5-year period of the CFC/ICCO/IPGRI project *"Cocoa Germplasm Utilization and Conservation: a Global Approach"*, a series of experiments was conducted using the detached pod test - spray method (DPT-SM) and the leaf disc test (LDT) to assess cocoa for resistance to *Phytophthora* infections. Data obtained in the project at the Cocoa Research Unit (CRU), The University of the West Indies, Trinidad and Tobago, provide an opportunity to assess the validity of DPT-SM and LDT. Between 1998 and 2003, 742 accessions were assessed in two trials for resistance to *Phytophthora* pod rot (Ppr) using the DPT-SM. Significant variation was observed in the reactions of the 742 accessions based on the disease rating scale, showing that the DPT-SM could effectively discriminate between various levels of resistance to Ppr. A chi-square test did not show any significant difference between the two trials of inoculation conducted. Furthermore, a high correlation coefficient ($r=0.80$, $P<0.001$) was obtained between the two trials, confirming that the results of DPT-SM are repeatable. Among the 742 accessions tested using the DPT-SM, 40 were assessed for resistance to Ppr in field conditions at the International Cocoa Genebank, Trinidad (ICG,T). Significant variation was observed among accessions and a higher level of susceptibility was observed in the third year of field observations (63%) than in the first (15%) and second (25%) years. This shows that absolute reliance could not be placed on a single year of field observations in determining clonal resistance to Ppr. The result of DPT-SM shows a higher correlation ($r=0.66$, $P<0.001$) with the average of years 1 to 3 field observations than with the result of each year of field observations, which varied from 0.44 to 0.59. It is possible that a stronger association may exist between the result of DPT-SM and the cumulative data on field observations for a period longer than three years. The overall correlation confirms the usefulness of DPT-SM as an effective method of assessing clonal resistance to Ppr and predicting field reaction in the long term. Significant differences were observed among 33 accessions screened for leaf resistance to *P. palmivora* using the LDT. This shows that the LDT could effectively discriminate between different levels of resistance among cocoa accessions. Correlations among the three inoculation trials conducted varied between 0.49 and 0.77. This shows that the results of LDT are repeatable. A poor correlation ($r=0.14$) was observed between the results of the LDT and DPT-SM, suggesting that the forms of resistance assessed by LDT and DPT-SM might be different. Since *Phytophthora* infects both pod and leaf, resistance in the two organs as assessed by LDT and DPT-SM may complement each other in breeding for a higher level of resistance to *Phytophthora* infections in cocoa.

Introduction

Phytophthora pod rot (Ppr) caused by *Phytophthora palmivora*, *P. megakarya*, *P. capsici* and *P. citrophthora* is one of the most prevalent and destructive diseases of cocoa (*Theobroma cacao* L.) (Iwaro *et al*. 1998). Global losses from Ppr are enormous and were estimated by

Opeke and Gorenz (1974) at about 20-30% of annual cocoa production. The development of high-yielding, resistant material is generally agreed to be the most effective and economic control method (Soria 1974; Rocha 1974; Iwaro *et al*. 2000a), but progress in this direction has been very slow, probably due to the narrow genetic base of most cocoa breeding programmes, a low level of resistance in base parents, and poor screening methods.

During the CFC/ICCO/IPGRI project "*Cocoa Germplasm Utilization and Conservation: a Global Approach*", two new inoculation methods were used in CRU for the assessment of *Phytophthora* infection in cocoa. The detached pod test - spray method (DPT-SM) was used for the assessment of pod resistance (Iwaro *et al*. 2000b), while the leaf disc test (LDT) was adopted for the assessment of leaf resistance (Nyassé *et al.* 1995). Data collected using the DPT-SM, LDT and field observations for resistance to Ppr provide an opportunity to assess the reliability of each inoculation method and to determine the relationship between the forms of resistance assessed by the different methods.

Materials and methods

Assessment of 742 accessions for resistance to Ppr using the DPT-SM

Between 1998 and 2003, the DPT-SM was used to evaluate 742 accessions in the International Cocoa Genebank, Trinidad (ICG,T) in two trials. DPT-SM was used to assess the reaction of fully-grown, unripe detached pods (about 4-5 months old) to *P. palmivora* (Iwaro *et al.* 2000b). As recommended by Iwaro *et al.* (2000b), two to four pods were tested per genotype in each of two trials conducted to confirm their reaction to *P. palmivora.* The levels of resistance of the inoculated pods were assessed using the disease rating scale below:

†Rating	Infection level
1	No visible lesion
2	1-5 localized lesions
3	6-15 localized lesions
4	15 localized lesions

† Based on the absence of visible lesions (rating 1) and the number of non-expanding (localized) lesions (rating 2-4)

‡Rating	Infection level
5	1-5 expanding lesions
6	6-15 expanding lesions
7	>15 expanding lesions
8	Fast expanding coalesced lesions

‡ Based on the number of expanding, countable lesions (rating 5-7) and expanding coalesced lesions (rating 8)

Data obtained were subjected to a chi-square test to assess the significance of differences between the two trials of inoculation. Furthermore, data were subjected to a Spearman rank correlation analysis to assess the repeatability of results between the two trials of inoculation. A distribution of scores was plotted to assess the pattern of variation and consequently the ability of the DPT-SM to effectively discriminate between different levels of resistance among the 742 accessions evaluated.

Field observations for resistance to *Phytophthora* pod rot

Among the 742 accessions screened for resistance to Ppr using DPT-SM, 40 genotypes were selected for field observations for resistance to Ppr. Five trees were selected for field observations of 32 genotypes, while observations were conducted on at least two trees for the remaining eight accessions with less than five trees per plot. An average of 24 pods per tree was assessed in each year of field observations. Each tree was observed monthly and the following variables were recorded:

- number of healthy pods (i.e. not showing any symptoms of Ppr), and
- number of pods with Ppr symptoms.

Field observations were carried out for three years between November 1998 and October 2001. The percentage of pods affected by Ppr was expressed as:

$$\frac{\text{Number of pods with Ppr symptoms}}{\text{Total number of pods produced}}$$

The data collected for each year of field observations were transformed using an arcsin square-root transformation and subjected to analysis of variance to determine the significance of the differences among clones and the years of field observations. Data were also subjected to Pearson correlation analysis to determine the relationship between the years of field observations. A distribution of scores was also plotted to assess the patterns of clonal reactions in each year of field observations. Subsequently, the data for each year of field observations and the results of DPT-SM were subjected to Spearman rank correlation analysis. In addition, correlation analyses were performed between the results of DPT-SM and averages of the first two years and three years of field observations.

Assessment of 33 accessions for leaf resistance to *P. palmivora* using the LDT

Thirty-three genotypes selected for the germplasm enhancement programme were multiplied clonally by top-grafting. Five replicates of each genotype were maintained for six months in the greenhouse. Leaves (inter-flush 2) were collected from each genotype and screened for resistance to *P. palmivora* using the leaf disc inoculation method (Nyassé *et al.* 1995). The experiment was repeated three times to assess the consistency of results. The levels of resistance of the inoculated leaf discs were assessed using the disease rating scale below:

Disease rating scale

0 no symptoms
1 very small localized brown or dark-brown penetration points
2 small penetration points with some connections between them
3 coalescence of brown spots forming intermediate-sized lesions
4 large, coalesced lesions containing lighter or darker brown coloured spots
5 large, uniformly expanding brown lesions

Data obtained from the three trials of inoculation were subjected to analysis of variance to determine the significance of differences among the 33 genotypes and the three trials of inoculation. Data obtained from the three trials were also subjected to Pearson correlation analysis to assess the consistency of results between trials. Further, a Spearman rank correlation analysis was conducted using data obtained from the DPT-SM and LDT on the 33 genotypes. A distribution of scores was plotted to assess the pattern of variation and consequently the ability of the LDT to effectively discriminate between the different levels of resistance among the 33 accessions.

Results and discussion

Assessment of genotypic differences among 742 accessions using the DPT-SM

A large proportion of the accessions tested were susceptible to the isolate of *P. palmivora* used in the experiment (Fig. 1). However, 91 accessions were found to be resistant (disease rating 1-3), 27 of which showed no visible lesions (disease rating 1). Among the other 64 resistant accessions, 30 had 1-5 localized lesions (disease rating 2), while 34 had 6-15 localized lesions (disease rating 3). Sixty-two more accessions were resistant to the spread of lesions (disease rating 4), since many localized lesions (>15) were observed on these. In contrast, a few expanding lesions (1-5 lesions) were formed on 79 accessions (disease rating 5). Two hundred and twelve accessions had 6-15 expanding lesions (disease rating 6), while 211 accessions had more than 15 expanding lesions (disease rating 7). Fast-expanding coalesced lesions were observed for 87 accessions. The distribution of scores for resistance to Ppr (Fig. 1) shows that the DPT-SM could effectively discriminate between the varying levels of resistance in the 742 accessions assessed.

The chi-square test did not show any significant difference between the two trials of inoculation conducted (χ^2=0.32, n.s.). In addition, a high correlation coefficient (r=0.80, P<0.001) was obtained between the two trials of inoculation conducted. This shows a close relationship between the results of the two trials of inoculation conducted, indicating that the results of DPT-SM are repeatable.

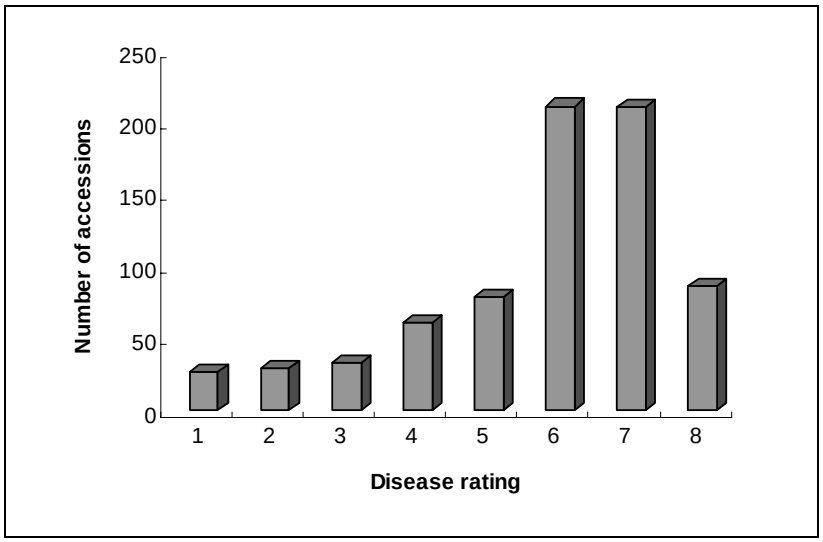

Fig. 1. Distribution of scores for resistance to *Phytophthora* pod rot among 742 accessions assessed by the detached pod test.

Field observations for resistance to *Phytophthora* pod rot on 40 accessions

Significant differences (P<0.001) were observed among the 40 genotypes assessed for resistance to Ppr by field observations (Table 1). Thirteen of the 40 accessions were found to be resistant (<10% pod rot), while 16 accessions were moderately resistant (10–25% pod rot) (Iwaro *et al.* 2005). Eleven accessions were classified as susceptible (>25% pod rot). Significant differences (P<0.001) were observed among the years of field observations and there was a significant interaction between clones and years (Table 1). There were differences in symptom expression among the years of field observations.

A correlation coefficient of 0.68 (P<0.001) was observed between the results of year-1 and year-2 field observations. However, lower correlation values were obtained between year-3 and year-1 (r=0.32, P=0.041) and year-3 and year-2 (r=0.35, P=0.025) field observations. A higher level of susceptibility was observed in the third year of field observations (63%) than in the first (15%) and second (25%) years (Fig. 2). This suggests that the predisposing factors for Ppr were unstable between the years of field observations. Data obtained from the Trinidad and Tobago Meteorological Services showed that the rainfall in November of the third year was higher than the amounts of rainfall in November of the first two years of field observations (Iwaro *et al.* 2005). This month marks the beginning of the main pod harvest season (November–February), and the high rainfall in November of the third year as well as the presence of a large number of mature pods may account in part for the increase in Ppr in the third year. This shows that absolute reliance should not be placed on a single year of field observations in determining clonal resistance to Ppr.

Table 1. Analysis of variance of clonal resistance to *Phytophthora* pod rot from field observations

Source	DF	Sum of Squares	Mean Square	*F*-Ratio	*P*-value
Clone	39	20.54	0.53	9.54	<0.001
Year	2	9.36	4.68	84.70	<0.001
Clone x Year	78	12.56	0.16	2.92	<0.001
Error	403	22.26			
Total (adjusted)	522	65.59			
Total	523				

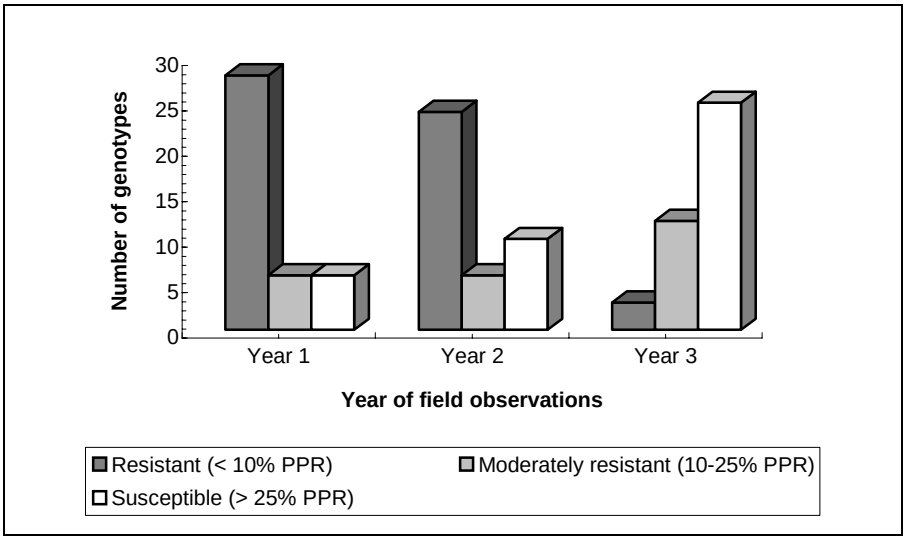

Fig. 2. Distribution of categories for resistance to *Phytophthora* from yearly field observations.

Relationship between field observations and the reaction of detached pods to *Phytophthora* pod rot

A correlation coefficient of 0.59 (P<0.001) was obtained between the result of year-2 field observations and DPT-SM. Lower correlation values were observed between DPT-SM and year-1 (r=0.55, P<0.001) and year-3 (r=0.44, P=0.005) field observations. The results of DPT-SM, however, show a higher correlation (r=0.66, P<0.001) with the average of field observations in years 1 to 3 (Fig. 3). It is possible that a stronger association may exist between the results of DPT-SM and the cumulative data on field observations for a period

longer than three years. The correlation (r=0.66, P<0.001) observed in this study confirms the usefulness of DPT-SM as an effective method of assessing clonal resistance to Ppr and predicting field reaction in the long term. Since field observations are labour-intensive and expensive to conduct on yearly basis, the DPT-SM offers a cheaper and more effective means of assessing clonal resistance to Ppr. Being a non-destructive inoculation method, the DPT-SM provides a suitable option for assessment of cocoa collections in genebanks. It is also a cost-effective method for use in cocoa breeding programmes.

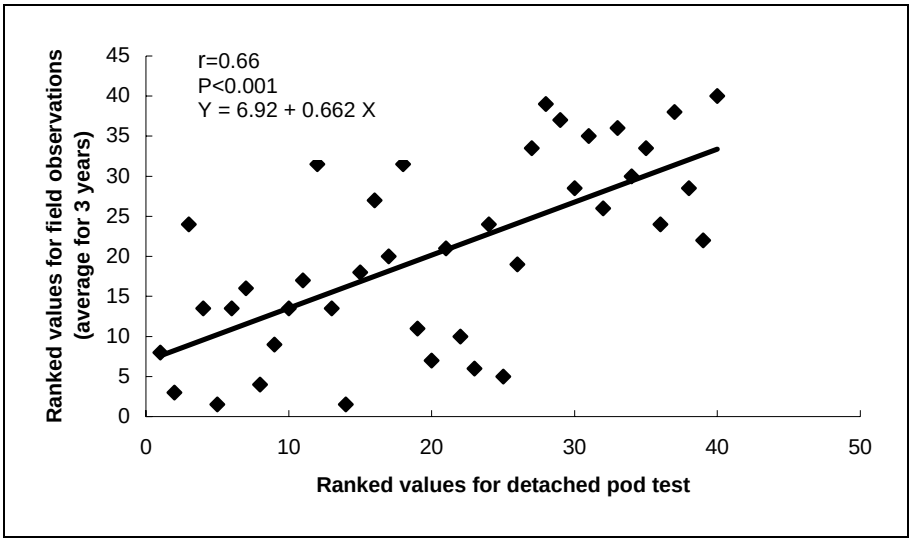

Fig. 3. Relationship between the average of 3 years of field observations and detached pod test for resistance to *Phytophthora* pod rot.

Assessment of genotypic differences among 33 accessions using the LDT

Figure 4 shows the distribution of scores for resistance among the 33 genotypes evaluated for leaf resistance using the LDT. None of the 33 genotypes was immune to the isolate of *P. palmivora* used in the experiment. Small penetration points with some connections between them (disease rating 2) were observed in two of the 33 genotypes, while 23 genotypes had coalescence of brown spots (disease rating 3). Large, coalesced lesions were observed in seven genotypes (disease rating 4), while the remaining one genotype had large, uniformly expanding brown lesions (disease rating 5). The results show that the LDT could effectively discriminate among varying levels of leaf resistance to *P. palmivora* in the 33 accessions assessed.

The three trials of inoculation were not significantly different from each other and a high correlation coefficient (r=0.77, P<0.001) was obtained between the results of trial-1 and trial-2. Lower correlation values were observed between trial-1 and trial-3 (r=0.49, P<0.001), and trial-2 and trial-3 (r=0.59, P<0.001). This shows that the results of LDT are repeatable. However, the range of correlation values obtained among trials (0.77-0.49) suggests some environmental impact on the methodology requiring an increase in replicated trials for a reliable assessment of genotypic effects.

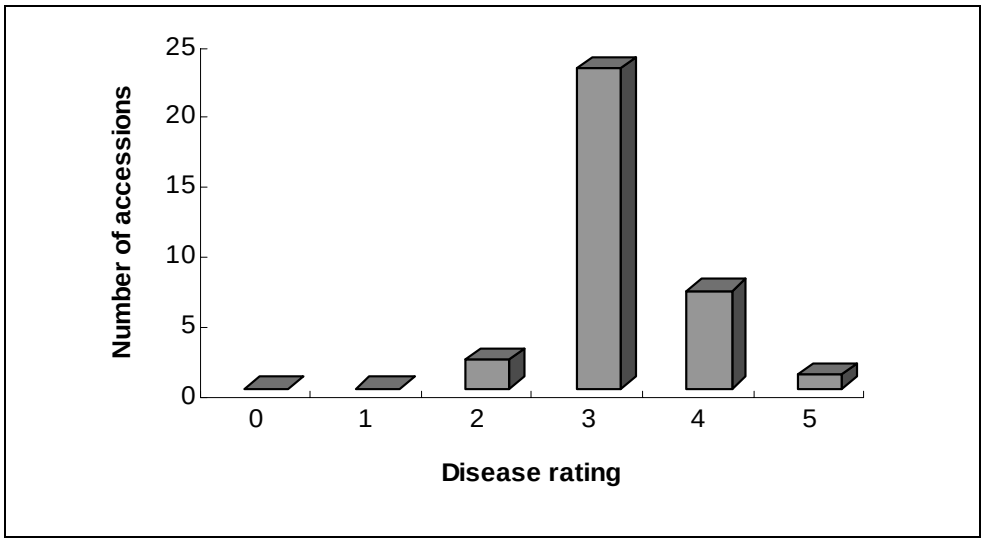

Fig. 4. Distribution of scores for resistance to *Phytophthora palmivora* among 33 accessions assessed by the leaf disc test.

Relationship between the results of the leaf disc test and the detached pod test

The correlation between the results of the LDT and DPT-SM for 30 accessions was poor and not significant (r=0.14). This might suggest that the forms of resistance assessed by LDT and DPT-SM could be different. It might however also be that the correlation is low as the most susceptible genotypes were not well represented in the sample of clones studied. Nyassé *et al*. (1995) had established a relationship between the results of LDT and field observations for resistance to Ppr. Iwaro *et al*. (1997) also established a relationship between pod and leaf resistance at the post-penetration stage of infection, but noted that the mechanism of resistance was different in the two organs at the penetration stage of infection. This may account in part for the poor correlation between the results of the LDT and DPT-SM. Further studies are required to fully understand the relationship between the forms of resistance assessed by the LDT and DPT-SM. Since *Phytophthora* infects both cocoa pods and leaves, the two forms of resistance assessed by LDT and DPT-SM may complement each other in breeding for a higher level of resistance to *Phytophthora* infections in cocoa.

It is hoped that the DPT-SM and LDT will facilitate the screening of more cocoa genotypes, the selection of promising resistant genotypes, and their use in breeding for the development of improved high-yielding, resistant cocoa varieties for commercial use.

Acknowledgements

We wish to thank the Common Fund for Commodities, the Biscuit, Cake, Chocolate and Confectionery Association of the United Kingdom and the Government of the Republic of Trinidad and Tobago for financial support. Our sincere gratitude goes to Prof. J. Spence, Mr R.A. Lass and Dr T.N. Sreenivasan for their support and interest in this work. We are also indebted to Ms V. Singh, Ms S. Bharath and Mr B. Latchman for assisting with data collection.

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Comparison of resistance to *Phytophthora* pod rot in a ring test using leaf disc inoculation at ten sites

C. Cilas

CIRAD-CP, TA 80/02, Avenue Agropolis, 34398 Montpellier cedex 5, France

Abstract

Phytophthora pod rot (Ppr) caused by different species of *Phytophthora* is the most widespread disease of cocoa. All producing countries are affected by this disease, albeit at varying intensities. Selecting resistant planting materials is therefore one of the priorities of genetic improvement. In order to select cocoa clones displaying resistance to *Phytophthora*, a ring test was organized in all the countries involved in the CFC/ICCO/IPGRI project. The different countries involved carried out a test on leaf discs for which the protocol had been drafted during earlier research. The test was used to classify different clones tested in each country according to their relative susceptibility to the *Phytophthora* species occurring in that country. An overview of the results is presented, focusing particularly on the interaction between the clones studied and the laboratory where the assessments were carried out. The interaction was statistically significant, indicating that clone classification was not identical for the different countries. Some clones, such as PA107 had stable reactions; others such as the Guianan clone GU255V had very unstable reactions: it was found to be very resistant in some laboratories and very susceptible in others. These interactions were due partly to the different isolates or species of *Phytophthora* used for the tests, or to different testing procedures used in different countries. Better standardization of the assessment protocols would nonetheless improve the reproducibility of the test. Mistakes in the identity of the clones in the various countries can also be a major factor for the significant clone x laboratory interaction.

Introduction

Phytophthora pod rot (Ppr) caused by several species of the genus *Phytophthora* cause substantial yield losses in the cocoa tree (*Theobroma cacao* L.); such losses are estimated at almost 30% worldwide (Lass 1985). Beans from affected pods are destroyed by the disease or are unfit for consumption.

Several species of this pathogen have been identified in the different production zones. The most widespread species is *P. palmivora*, which exists in virtually all producing countries. In Cameroon, recent studies have revealed the existence of a single species, *P. megakarya* (Nyassé 1997), which is considered to be the most aggressive species (Brasier and Griffin 1979). *P. megakarya* is also found in Nigeria, Togo, Ghana, and in western Côte d'Ivoire. On the American continent, the species *P. capsici* has been detected in numerous production zones, and the species *P. citrophthora* exists in Brazil. Lastly, the species *P. arecae* has been detected in South-East Asia and the Pacific region.

Selecting cocoa trees that are less susceptible to black pod disease (*Phytophthora* pod rot or Ppr) therefore remains a priority research objective. Despite a great deal of work (Tarjot 1969; Blaha and Lotodé 1976; Berry and Cilas 1994), the search for cocoa trees displaying total resistance to this disease has failed. Many authors have suggested that the differences in reaction to *Phytophthora* spp. were due to partial, probably polygenic resistance (Partiot 1975; Blaha and Lotodé 1977). However, such resistance displays additive type of heredity (Tan and Tan 1990; Cilas and Despréaux 2004). Different ways of testing planting material have been tried (Blaha 1974); observation of field performance under natural infection conditions

and artificial inoculation tests on pods or leaves remain the main methods adopted. A good relation between the test on leaf discs and the rot rate observed in the field has been found at the family level (Nyassé *et al*. 2002), making it possible to use this early test in selection processes, and thereby speed up selection cycles, whose length is often the main obstacle to genetic progress in perennial species.

Using leaves to develop a non-destructive early test that could be repeated virtually at will on the same plants is a particularly attractive idea, particularly as young leaves can be attacked naturally by *Phytophthora*, especially *P. palmivora*, and the histological structure of the underside of leaves is similar to that of the superficial layers of pods (Van der Vossen 1997).

Several teams had already examined the possibility of using cocoa tree leaves to predict the plant's degree of resistance to *Phytophthora* (Tarjot 1972; Tondje *et al*. 1988), but leaf tests only became operational once Nyassé *et al*. (1995) and Iwaro *et al*. (1997) developed and demonstrated the merits of their methods (Nyassé *et al*. 2002). The tests proposed by those authors, which were pre-eminently early tests that could be carried out in the nursery and were easy and cheap to apply, made it possible to shorten considerably cocoa breeding cycles by selecting resistant plants at a very young age from among the progenies created in pre-breeding programmes.

Although a test developed in one country cannot usually be directly transposed to another country, where conditions are not identical (different species of *Phytophthora* or isolates differing in aggressiveness, different environmental conditions in the nurseries or fields, and different laboratory conditions), it is often requested in international projects that the tests used be as standardized as possible, in order to compare results from one country to the other.

In this work we therefore present the results obtained with the leaf disc test on clones tested in ten countries. This ring test was used to assess the resistance of candidate clones for selection with great reliability; it was also possible to assess interactions between the cocoa clones and the laboratories where the tests were applied.

Materials and methods

The leaf test basic method was described in the project protocol. This method was followed as well as possible, but somewhat adapted in the various laboratories. This basic method is briefly recalled below.

Choice of leaves

The leaf stages used were generally defined using the nomenclature of Greathouse *et al*. (1971). Leaves of the Interflush 1 stage correspond to leaves of mature size but supple and pale green or red depending on the planting material, those of the Interflush 2 to leaves of mature size, but dark green and borne on a green stem, while leaves of the Interflush 3 stage correspond to leaves of mature size, dark green in colour, but borne on a stem undergoing lignification. It is not rare to see *Phytophthora* on young supple leaves under particularly wet conditions, but not on mature leaves. The two stages, Interflush 2 and Interflush 3, made it possible to differentiate between clones for the duration of the experiment, and were recommended by Thévenin and Motilal (2000) for the former and by Nyassé *et al*. (1995) for the latter.

Disc sampling zone

In order to standardize the leaf disc assessment method, a study was conducted to compare different leaf disc sampling zones. As stomata open first in the apical zone of the leaf,

differences in reactivity to the pathogen were suspected between the different zones of the leaf.

Tahi (2003) therefore studied how the leaf disc sampling zone (part near the petiole, median section of the lamina, and part near the apex) affected symptom expression, with the following three clones: PA150, T60/887 and NA79. No difference was detected between the three sampling zones, which simplified the disc sampling method.

Disc preparation

Discs need to be prepared as quickly as possible after leaf harvesting to prevent any deterioration in the leaves. Using a semi-automatic device is also recommended for cutting the discs, to guarantee uniformity and quality. Using such a device saves valuable time when numerous plants have to be assessed in the same test, which is not an insubstantial factor in preventing plant material from drying when preparation takes too long.

The bottom of the trays used for the experiment was covered with a damp sponge on which the leaf discs were placed underside up, as the underside where the stomata are found is the most receptive to infection (Iwaro *et al*. 1997).

Inoculation and incubation

The inoculum was prepared over a period of 10 days including a mycelium growth phase in the dark on V8 medium and a sporocyst formation phase in alternating light and dark. The culture incubation temperatures for infectious propagule growth and production can vary from one species of *Phytophthora* to another (Ducamp 2000). To release zoospores, the cultures needed a thermal shock: the cultures were submerged in sterile distilled water and refrigerated at 4°C for 15 minutes, and then placed at room temperature for 30 to 60 minutes.

Several studies were undertaken, including the work by Thévenin and Motilal (2000) examining how zoospore concentration affected symptom expression. Inoculations with *P. palmivora* carried out with 10 µl drops per disc showed that the resulting symptom intensity generally increased as the zoospore concentration rose from 100 000 to 500 000 zoospores/ml. However, the differences between the highest two concentrations were not significant.

Incubation was carried out in the dark for the duration of the experiment (Thévenin and Motilal 2000). The temperatures adopted for incubation were 22°C for *P. megakarya*, 25°C for *P. palmivora* and 28°C for *P. capsici* "cacao". When comparing the level of aggressiveness of several species, a temperature of 25°C was favoured as it enabled each of the species to express a degree of aggressiveness approaching its maximum level.

Assessment of symptoms

The two methods developed virtually simultaneously by Nyassé *et al*. (1995) and Iwaro (1995) used symptom assessment scales based on different criteria: the development of symptoms from localized penetration points to the formation of a necrotic patch in the first case, and the number of penetration points or lesions and the area of the necrosis in the second case. It seemed worthwhile comparing those two scales; however, as it was not possible with leaf discs to measure the area of the lesion, the following two scales were compared by Thévenin and Motilal (2000):

Scale "A"

0	absence of symptoms
1	isolated penetration points
2	network of points
3	reticulated patch
4	mottled patch
5	true patch

interpreted as follows:

0	absence of symptoms
1	localized penetration points
2	small developing lesions, sometimes touching each other
3	merging lesions
4	more or less uniform lesion, light brown, sometimes still with some isolated lesions
5	large uniform lesion, typical dark brown colour of necrosis

Scale "B"

0	no lesions
1	1-19 localized lesions
2	20 or more localized lesions
3	1-19 expanding lesions
4	20 or more expanding lesions
5	merged lesions

The authors found that method "B" uniformly and significantly gave higher scores than method "A". In fact, this was not surprising since method "A" was based on the shape of the lesions and their gradual development, whereas method "B" gave a maximum score of "5" as soon as the lesions could not be distinguished from each other. However, clone classification was the same irrespectively of the method used, particularly 3 days after inoculation when the symptoms had yet to evolve.

Note that at CIRAD in Montpellier, a software (OPTIMAS) combined with an image analyzer is in the process of being optimized so as to be able to precisely quantify the necrotic areas and the number of penetration points per inoculated disc, 3 days after inoculation to acquire information on resistance to penetration, and 7 days after inoculation for information on resistance to symptom development.

Planting material studied

Several clones widely distributed in the different producing countries were chosen for the ring test. We analyzed 25 clones that had been tested in at least 5 countries. The 25 clones distributed in 10 countries formed an incomplete grid (Table 1). Nonetheless, this design made it possible to study "clone x country" interaction based on an incomplete factorial design.

Statistical analysis

Analyses were carried out by the collaborating institutions in each of ten countries to compare the resistance of the different clones using local isolates of *Phytophthora* (Table 1). Those results were used to carry out a general comparative analysis, the main objective being to estimate "clone x laboratory" interactions, in order to judge the reproducibility of the tests from one laboratory to another, with different environmental conditions and different isolates.

Table 1. List of clones tested per country and institute

Clone	Country									
	Cameroon (IRAD)	Côte d'Ivoire (CNRA)	Ghana (CRIG)	Nigeria (CRIN)	Brazil (CEPEC)	Trinidad (CRU)	Venezuela (INIA)	Malaysia (MCB)	PNG (CCI)	France (CIRAD)
AMAZ15-15	X	X	X	X	X	X	X	X	X	X
AMAZ5-2		X	X	X	X					X
APA4			X	X	X		X		X	X
BE10	X	X	X	X	X	X	X		X	X
CATIE1000		X		X	X	X			X	X
EET59		X	X	X	X	X	X		X	X
EQX3360-3	X	X	X		X	X	X		X	X
GU255V	X	X			X	X	X		X	X
ICS1	X	X		X	X	X			X	X
IFC5	X	X	X	X					X	X
IMC47		X	X	X	X	X	X		X	X
LCTEEN46	X	X		X		X	X		X	X
MAN15-2	X	X	X	X		X	X	X	X	X
Mocorongo		X	X	X	X		X	X	X	X
MXC67	X	X		X		X	X	X	X	X
PA107	X	X	X	X		X	X	X	X	X
PA120		X	X	X		X	X		X	X
PA150		X	X	X		X	X	X	X	X
P7		X		X				X	X	X
Playa Alta 2	X	X		X		X	X	X		
SCA6		X	X	X	X	X	X	X	X	X
SPEC54-1	X	X	X	X		X	X			X
T85/799		X	X	X				X	X	X
UF676		X		X				X	X	X
VENC4-4		X	X	X		X	X		X	X

Results

Analyses of the assessments made in the different laboratories led to classifications being established for each country. The stability of the assessments over those different countries was studied with an incomplete factorial design with 25 clones assessed in at least 5 countries. In fact, the overall analysis of the results made it possible to estimate a country effect, a clone effect, and an effect of interaction between the two main effects. The factorial analysis was possible even though the design was incomplete, i.e. not all the clones were tested in all the countries. The results of this analysis of variance are shown in Table 2.

Table 2. Overall analysis of variance for country, clone and interaction effects

Source	DF	Mean Squares	F	P
Country	9	34.41	97.47	<0.001
Clone	24	0.82	2.33	<0.001
Country x Clone	133	0.70	2.00	<0.001
Error	1044	0.35		

The "clone x country" interaction means that classification of the clones according to their degree of susceptibility varied depending on the country where the tests were applied. Those interactions may have been caused either by an interaction with the *Phytophthora* species or isolates used, or an interaction with the various environmental effects linked to the conditions under which the test was carried out in the different laboratories. Misidentification of clones in the different countries could also lead to the strong "clone x country" interaction. A partial answer can be found in the interactions detected with several species of *Phytophthora* in similar tests carried out in the Montpellier laboratory (Ducamp 2000): "clone x *Phytophthora* species" interactions had been detected, but the F value of that interaction was much lower than for the clone effect. In this ring test, two sources of interaction were combined: "clone x *Phytophthora* species" interactions and

"clone x laboratory" interactions. Nevertheless, an overall comparison of the clones is proposed with a comparison of multiple means test (Table 3).

The overall classification did not take into account their varying degrees of stability over the set of countries in which the tests were performed. It had already been found that some clones displayed more stable reactions than others during inoculations. An examination of the clone classification, from the most resistant to the most susceptible in each country, revealed clones with stable reactions and clones with variable reactions depending on the countries (Table 4).

Some clones had stable assessments over the different countries, such as PA107 which was judged resistant, or clones MXC67 or UF676 which appeared to be susceptible in all the countries where they were tested. Conversely, some other clones showed highly variable responses depending on the country, such as IMC47, GU255V, IFC5 or ICS1.

Table 3. Comparison of the 25 clones in the 10 countries

Clone	Means	Uniform groups, Newman & Keuls (5%)	Clone	Means	Uniform groups, Newman & Keuls (5%)
AMAZ5-2	2.07	a	BE10	2.85	bcde
SCA6	2.38	ab	PA120	2.88	bcde
CATIE1000	2.43	abc	APA4	2.93	bcde
T85/799	2.46	abc	SPEC54-1	2.95	bcde
P7	2.59	bcd	MXC67	2.98	cde
IMC47	2.67	bcd	EQX3360-3	2.99	cde
PA107	2.75	bcde	VENC4-4	3.00	cde
MAN15-2	2.79	bcde	LCTEEN46	3.00	cde
PA150	2.80	bcde	EET59	3.16	de
IFC5	2.81	bcde	Mocorongo	3.16	de
AMAZ15-15	2.81	bcde	Playa Alta 2	3.28	e
ICS1	2.83	bcde	UF676	3.30	e
GU255V	2.84	bcde			

Table 4. Clone classification, from the most resistant to the most susceptible, based on assessments obtained in each country

Cameroon	Côte d'Ivoire	Ghana	Nigeria	Brazil	Trinidad	Venezuela	Malaysia	PNG	France
PA107	SCA6	SPEC54-1	SPEC54-1	CATIE1000	MAN15-2	SCA6	PA107	VENC4-4	IMC47
Playa Alta 2	SPEC54-1	AMAZ5-2	P7	AMAZ5-2	PA107	GU255V	P7	T85/799	GU255V
AMAZ15-15	AMAZ15-15	BE10	AMAZ5-2	SCA6	GU255V	MAN15-2	MAN15-2	PA107	SCA6
BE10	GU255V	IFC5	T85/799	BE10	SPEC54-1	IMC47	Playa Alta 2	EET59	PA120
MAN15-2	T85/799	ICS1	EET59	GU255V	Playa Alta 2	PA107	SCA6	Mocorongo	AMAZ15-15
ICS1	CATIE1000	PA107	LCTEEN46	Mocorongo	BE10	APA4	Mocorongo	BE10	P7
EQX3360-3	AMAZ5-2	AMAZ15-15	PA107	EQX3360-3	VENC4-4	Mocorongo	T85/799	CATIE1000	T85/799
MXC67	BE10	SCA6	PA120	AMAZ15-15	CATIE1000	BE10	MXC67	MAN15-2	MAN15-2
UF676	PA107	IMC47	SCA6	IMC47	SCA6	EET59	AMAZ15-15	IMC47	APA4
LCTEEN46	P7	PA120	CATIE1000	APA4	PA120	AMAZ15-15	UF676	SCA6	VENC4-4
GU255V	Mocorongo	T85/799	IFC5	EET59	IMC47	PA120		IFC5	CATIE1000
SPEC54-1	MAN15-2	APA4	Playa Alta 2	Playa Alta 2	LCTEEN46	VENC4-4		SPEC54-1	PA107
IFC5	IMC47	EET59	Mocorongo	ICS1	MXC67	LCTEEN46		AMAZ15-15	IFC5
	ICS1	VENC4-4	VENC4-4		AMAZ15-15	SPEC54-1		PA120	SPEC54-1
	Playa Alta 2	GU255V	MAN15-2		EET59	Playa Alta 2		EQX3360-3	EET59
	LCTEEN46	MXC67	BE10		ICS1	EQX3360-3		MXC67	LCTEEN46
	IFC5	MAN15-2	ICS1		EQX3360-3	MXC67		APA4	MXC67
	MXC67	UF676	UF676					P7	BE10
	EET59	Mocorongo	AMAZ15-15					ICS1	EQX3360-3
	EQX3360-3		MXC67					UF676	UF676
			APA4					PA150	
								LCTEEN46	
								GU255V	

Conclusion and discussion

The assessment of plant resistance to diseases always raises measurement problems. Assessing cocoa trees for their resistance to Ppr caused by different species of *Phytophthora* clearly illustrates that difficulty. Assessment in the field, under natural infestation conditions, can be biased by the micro-environmental conditions in which the epidemic develops. In addition, such field assessment requires the production of a minimum number of pods, which is not often achieved each year on each tree, in order to carry out reliable measurement of the affected pod rate. The recommendation often made is therefore to estimate rot rates per tree over at least 3 to 4 harvesting years. However, year-effects may disrupt measurement: indeed, if the yields of a tree, expressed as the total number of pods formed, have been greater in a year of strong disease incidence, it will undoubtedly be classified as more susceptible than it really is. Even if that year-effect can be controlled by appropriate statistical analyses, by trying to control the spatiotemporal effects of epidemics as much as possible, the genetic value of a tree in the field can only be known 5 or 6 years after planting. As artificial inoculation tests can be carried out at early stages, they are a way of bypassing the time needed for field assessments and of more effectively controlling the experimental conditions. However, it is necessary that those tests be reliable and accurate enough and that their good correlation with resistance in the field be established. A resistance test also needs to be reproducible, i.e. it must lead to identical classifications in time and space. The results presented in our work, based on a ring test performed with 32 clones assessed in 10 countries, revealed that "clone x country" interactions were significant and almost as important as the clone effects. Those interactions partly came from "clone x *Phytophthora* species and/or isolate" interactions, but substantial laboratory effects must also have disrupted the results. Nevertheless, some clones remained stable over the different countries for their susceptibility score. However, it seems necessary to continue standardizing the tests to reduce the laboratory effects, and especially "clone x laboratory" interaction, with a view to obtain a more accurate measurement of the genetic value of material tested for resistance to *Phytophthora*.

Acknowledgements

The author wishes to acknowledge the scientists at the collaborating institutions for making available their data on the resistance of the "international clones" to local isolates of *Phytophthora* as evaluated by using the leaf disc test. These scientists are: S. Agbeniyi (CRIN, Nigeria), G. Blaha (CCI/CIRAD, Papua New Guinea), C.L. Bong and M.J. Ahmad Kamil (MCB, Malaysia), M. Ducamp and D. Paulin (CIRAD, France), I. Kébé (CNRA, Côte d'Ivoire), E.D. Luz (CEPEC, Brazil), S. Nyassé (IRAD, Cameroon), J. Opoku (CRIG, Ghana), D. Parra (INIA, Venezuela) and J.-M. Thévenin (CRU/CIRAD, Trinidad).

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Isolate by clone interaction as assessed with the leaf disc test in Montpellier

D. Paulin[1], M. Ducamp[1], K. Vezian-Bonnemayre[1] and A.B. Eskes[2]

[1] CIRAD, Avenue Agropolis, 34398 Montpellier cedex 5, France
[2] IPGRI/CIRAD, c/o INIBAP, Parc Scientifique Agropolis II, 34397 Montpellier cedex 5, France

Abstract

Multi-site clone trials were planted by each of the CFC/ICCO/IPGRI project partners. For this, 32 clones, 20 of which were common, were delivered to ten countries in 1998 and 1999 by the quarantine centres at CIRAD in Montpellier and at the University of Reading. CIRAD in Montpellier evaluated the level of resistance to black pod disease (*Phytophthora* pod rot or Ppr) in 30 of those clones by the leaf inoculation test with 30 different isolates of *P. palmivora*, *P. megakarya*, *P. capsici* and *P. parasitica* originating from the ten cocoa-producing countries taking part in the project. There were four series of inoculations for each clone/isolate combination, with 10 leaf discs per series. The different isolates and species used were characterized by molecular markers and by *in vitro* observations, which showed that the degree of isolate aggressiveness was affected by the culturing and inoculum preparation conditions. The subsequent inoculation results revealed significant isolate and clone effects. Classification of isolates according to their degree of aggressiveness and of clones according to their resistance level is given in detail. The most resistant clones were IMC47, PA120, GU255V, GU307V, NA33, SCA6, AMAZ15-15, MAN15-2 and P7. The most susceptible clones were LAF1, N38, EET59, VENC22-6, BE10, LCTEEN46, MXC67 and EQX3360-3. For the species *P. palmivora*, the isolates from Trinidad and an isolate from Côte d'Ivoire were more aggressive than the isolates from Papua New Guinea, Ecuador, Ghana and Malaysia. For the species *P. megakarya*, the isolates from Nigeria were more aggressive than those from Cameroon and Ghana. For the species *P. capsici* the isolates from Trinidad were more aggressive than those from Malaysia. The effects of interaction between clones and isolates were generally less than the clone and isolate effects. The classification of clone resistance was significantly correlated for 85% of the comparisons between isolates. This relative stability of resistance in relation to the different species and isolates of *Phytophthora* meant that varieties with a wide spectrum of resistance could be selected using only one isolate in resistance tests.

Introduction

One of the main purposes of the CFC/ICCO/IPGRI project was to increase the efforts to assess cocoa genetic resources (*Theobroma cacao* L.) for resistance to diseases and insects (Eskes *et al.* 1998). Under this project, 32 "international clones" were chosen and distributed to ten producing countries from quarantine centres at the University of Reading (UK) and CIRAD in Montpellier (France). Those clones were chosen on the basis of available information concerning their degree of resistance to *Phytophthora* in the field, their genetic origin and their yield level. The degree of resistance in the clones was assessed at Montpellier with isolates of *P. megakarya*, *P. palmivora*, *P. capsici* and *P. parasitica* from the ten countries, using the leaf disc inoculation test developed and validated before the project began (e.g. Nyassé *et al.* 1995; Tahi *et al.* 2000).

The main aims of this study were:

- Characterization of isolates and species of *Phytophthora* from the ten producing countries by molecular markers (internal transcribed spacer (ITS) sequences);
- Comparison of the resistance levels of the international clones and of the aggressiveness levels of the *Phytophthora* isolates, and an analysis of host and parasite interactions;
- Improvement of resistance screening conditions depending on the species studied; and
- Comparison of the resistance levels of the international clones obtained at Montpellier with those obtained in the countries from which the isolates came, using the same test on leaf discs (results not shown here).

Materials and methods

Fungal material

In this study, 31 isolates of *Phytophthora* from 11 countries were received at CIRAD in Montpellier (Table 1): 19 *P. palmivora*, 7 *P. megakarya*, 4 *P. capsici* and 1 *P. parasitica*. These isolates were sent by scientists working in the CGC/ICCO/IPGRI project. Confirmation that one isolate belongs to the latter species was obtained by studying their ITS sequence in accordance with the protocol established by Lee and Taylor (1992). The mating type of each isolate was determined by *in vitro* comparison with compatible reference isolates existing in the Montpellier collection. The speed of mycelium growth was studied for each isolate on V8 nutritional medium in Petri dishes incubated in the dark at different temperatures. There were five replicates with three dishes per isolate for each replicate. The number of zoospores produced was determined after 10-day culture on V8 medium with 3 days in the dark and 7 days of alternating 12 h-light and 12 h-dark. There were ten replicates with three dishes per isolate and per temperature for each replicate.

Table 1. Characteristics of the *Phytophthora* isolates pathogenic on cocoa used in the study

Species	Origin	Mating type
P. palmivora*		
PNG1	Papua New Guinea	A2
PNG2	"	A2
PNG3	"	A2
PNG4	"	A2
PNG5	"	A1
CIV99.1	Côte d'Ivoire	A2
CIV99.2	"	A2
TRI1	Trinidad and Tobago	A2
TRI17	"	A2
TRI18	"	A2
NGRpal	Nigeria	A2
LKM45	Malaysia	A2
LKM54	"	A2
GH18	Ghana	A2
P517	"	A2
EQ1	Ecuador	A2
EQ2	"	A2
BR23	Brazil	A2
VEN1	Venezuela	A2

Species	Origin	Mating type
P. megakarya*		
ASH36 = GH19	Ghana	A1
NGR16	Nigeria	A2
NGR20 = NGRmeg	"	A1
NS328	Cameroon	A1
NS331	"	A1
NS341	"	A1
NS269	"	A1

P. capsici*		
TRI3	Trinidad and Tobago	A2
TRI19	"	A1
LKM43	Malaysia	A2
BR26	Brazil	A1

P. parasitica*		
LKM07	Malaysia	?

* confirmed to belong to this species by an ITS sequence study

Plant material

The 32 international clones in the CFC/ICCO/IPGRI project (Eskes *et al*. 1998) were propagated in Montpellier by budgrafting: IMC47 (resistant control), LAF1 (susceptible control), GU255V, GU307V, GU175V, T85/799, T79/501, PA120, PA150, NA33, APA4, VENC4-4, VENC22-6, EET59, GF24, SNK64, SNK413, IFC5, ICS1, Mocorongo, LAF1, N38, CAT1000, P7, SCA6, AMAZ15-15, MAN15-2, SPEC54-1, PA107, MCX67, BE10, LCTEEN46 and EQX3360-3.

Inoculation protocol

The resistance levels of the clones and the aggressiveness of the isolates were assessed by inoculating leaf discs with *Phytophthora* spores (Nyassé *et al*. 1995). Seven inoculation assays were carried out to test the 32 clones and 31 isolates. For each assay, 4 series (inoculations on different dates) were carried out. For one series, 5 leaves were inoculated per clone with 5 to 11 isolates. For each clone/isolate pair, at least 10 discs were inoculated per series, with 2 discs of each of the 5 leaves, distributed over 5 trays (replicates), with 2 discs per tray for each clone/isolate pair. In all, at least 40 discs were therefore used to assess clone resistance to an isolate.

For the complete set of assays, the leaves of two control clones, IMC47 and LAF1, were inoculated with the two control isolates TRI1 and P517. The inoculated discs on trays were incubated in the dark at 25°C. Symptoms were scored 7 days after inoculation, using the patch type scale developed by Nyassé *et al*. (1995), and completed by observing the size of the patches, varying from 1 to 5, for patch types high in the scale (3, 4 and 5). The final average score (for patch type and patch size) obtained for each plant/pathogen pair was called "INDE7". These values were only corrected by the values of the controls in assays where the means of the controls were substantially different from the other assays.

Statistical analyses

Statistical analyses were carried out with SAS software. The means per assay were compared by the Newman and Keuls test, at 5% probability.

Results

Effect of culture conditions on inoculum quality

Different parameters were examined during this study, notably the effect of temperature on mycelium development and the ability of the different species of *Phytophthora* to sporulate (Table 2). For *P. palmivora*, mycelium developed best between 25 and 30°C, with an optimum sporulation capacity between 25 and 28°C. For *P. capsici* and *P. parasitica*, mycelium growth seemed indifferent to temperature, but zoospore formation was optimal between 25 and 28°C. For *P. megakarya*, there was little mycelium development over 28°C and its ability to sporulate was poor. No zoospore formation was obtained for *P. megakarya* at 30°C. The isolates of *P. palmivora* from Trinidad had the greatest sporulation capacity between 25°C and 28°C. The isolates of *P. capsici* displayed the fastest mycelium growth.

Table 2. Effect of temperature on mycelium growth rate and on zoospore production of isolates of *Phytophthora* pathogenic on cocoa

Species	Mycelium growth rate (mm/3 days)			Number of zoospores produced/ml (x 10^3)			
	20°C	25°C	30°C	20°C	25°C	28°C	30°C
P. palmivora							
PNG1	39.5	45	53	84	200	450	210
PNG2	25	42	37	56	201	248	94
PNG3	28	46	45	108	303	346	103
PNG4	38	39.5	39.5	115	800	540	309
PNG5	47	56	65	69	210	350	223
CIV99.1	48	55	54	53	315	314	108
CIV99.2	48	63	65	-	-	-	-
TRI1 (T)	44	53	36	107	1245	807	210
TRI17	39	49	49.5	84	1006	951	354
TRI18	38	43	49	92	995	834	359
NGRpal	34	49	45	-	-	-	-
LKM45	40	59	53	63	284	243	120
LKM54	44	59	56	-	-	-	-
GH18	44	60	57	80	225	211	105
P517	39	53	57	-	-	-	-
EQ1	39	48	52	71	216	242	143
EQ2	33	53	47	-	-	-	-
BR23	42	55	57	63	443	218	111
VEN1	30	38	35	-	-	-	-
P. megakarya							
GH19	32	37	18	151	422	103	0
NRG16	37	38	16	186	520	59	0
NRGmeg	40	44	21	108	466	101	0
NS328	41	48	14	125	338	92	0
NS331	45	54	27	-	-	-	-
NS341	39	46	25	-	-	-	-
NS269	54	69	33	202	673	282	0
P. capsici							
TRI3	53	56	57	54	453	704	250
TRI19	55	67	61	44	326	682	225
LMK43	53	60	64	24	284	364	159
BR26	50	64	65	18	449	577	304
P. parasitica							
LMK07	32	37.5	38	21	301	484	206

Statistical analysis of clone and isolate effects:

Seven inoculation assays were performed with a choice of different clones and isolates depending on the availability of spores and of leaves at the right stage. An analysis of variance (Table 3) and classification of the factors were therefore carried out for each assay separately. The general means obtained were uniform depending on the series (from 2.15 to 2.94).

The results of the variance analyses revealed highly significant clone and isolate effects for each assay. Two clone/isolate control pairs were used in each assay: IMC47, as the resistant clone, and LAF1, as the susceptible clone, inoculated with TRI1, as the aggressive isolate, and P517, as the low aggression isolate. The isolate effects were often greater than the clone effects. In series 6, the F value was very high due to the very low aggressiveness of isolate LKM07.

Effect of interaction between clones and isolates

There was no significant effect of interaction between clones and isolates in the first three assays (Table 3). However, the interaction effect was significant for the other assays, though with low F values compared with the F values for clones and isolates.

Table 3. Analysis of variance of clone, isolate and interaction effects for the seven inoculation assays (32 clones inoculated by 30 isolates)

	Assay 1	Assay 2	Assay 3	Assay 4	Assay 5	Assay 6	Assay 7
Mean	2.8	2.15	2.60	2.94	2.37	2.41	2.55
Total variance	1.37	1.36	1.30	0.73	0.61	1.62	1.24
DF clone/isolate	19 / 10	14 / 10	16 / 8	13 / 9	16 / 9	17 / 8	14/4
F clone	16**	12**	13**	230**	95**	57**	17**
F isolate	20**	17**	14**	111**	138**	744**	88**
F interaction	0.7	0.7	0.9	4**	4**	6**	2**

** = significant at P<0.01

The most resistant clones were Upper Amazon or Guianan materials: IMC47, PA120, GU255V, GU307V, NA33, SCA6, AMAZ15-15, MAN15-2 and P7. The most susceptible clones were LAF1, N38, EET59, VENC22-6, BE10, LCTEEN46, MXC67 and EQX3360-3. The clone classifications were generally fairly uniform between the assays. However, classification of SNK413 differed depending on the assays. In the first assay, this clone was classified as susceptible when inoculated with fairly aggressive isolates of *P. palmivora*. In the second assay, SNK413 was classified as more resistant due to its good level of resistance to the three isolates of *P. capsici* (Table 4).

Table 4. Classification of clones for their level of resistance to *Phytopthora* sp. by the leaf inoculation test (in order of most resistant to most susceptible) (control clones in bold)

Assay 1		Assay 2		Assay 3		Assay 4		Assay 5		Assay 6		Assay 7	
IMC47	**a**	PA120	a	**IMC47**	**a**	**IMC47**	**a**	**IMC47**	**a**	**IMC47**	**a**	**IMC47**	**a**
GU255V	b	**IMC47**	**a**	GU255V	b	SCA6	a	AMAZ15-15	b	P7	b	GU255V	ab
T85/799	b	GU255V	a	PA120	c	AMAZ15-15	b	SCA6	b	AMAZ15-15	b	PA120	b
PA120	b	GU307V	a	NA33	c	P7	b	P7	b	SCA6	b	NA33	b
GU307V	b	NA33	ab	GU307V	c	MAN15-2	c	MAN15-2	c	MAN15-2	c	GU307V	b
NA33	bc	SNK413	abc	VENC4-4	c	SPEC54-1	d	IFC5	d	T85/799	c	VENC4-4	bc
APA4	bcd	APA4	bcd	VENC22-6	c	CAT1000	de	PA107	d	CAT1000	d	APA4	bc
GU175V	cde	GU175V	bcd	GU175V	c	PA107	ef	T85/799	de	T79/701	de	GU175V	c
VENC4-4	cde	PA150	bcd	PA150	cd	MXC67	fg	CAT1000	ef	**LAF1**	**de**	**LAF1**	**c**
EET59	e	**LAF1**	**bcd**	GF24	cd	N38	fg	T79/501	efg	PA107	de	ICS1	c
GF24	e	VENC4-4	bcd	SNK413	cd	BE10	gh	**LAF1**	**fg**	IFC5	de	EET59	c
PA150	e	VENC22-6	cd	SNK64	cd	**LAF1**	**gh**	LCTEEN46	h	SPEC54-1	de	PA150	cd
SNK64	ef	EET59	de	**LAF1**	**cd**	LCTEEN46	h	SPEC54-1	h	BE10	de	SNK413	cd
IFC5	ef	ICS1	e	APA4	cd	EQX3360-3	i	EQX3360-3	h	MXC67	e	SNK64	cd
ICS1	ef	SNK64	e	ICS1	cd			MXC67	i	LCTEEN46	e	VENC22-6	d
T79/501	ef			EET59	d			BE10	i	GF24	e		
VENC22-6	ef			N38	d			N38	j	EQX3360-3	f		
SNK413	ef									N38	f		
Mocorongo	ef												
LAF1	**f**												

Classification of the isolates is shown in Table 5. In the second inoculation assay, control isolate TRI1 displayed less aggressiveness than in the other assays, particularly with the susceptible clone LAF1, whilst in the other assays this isolate had a higher aggressiveness level than P517. For the species *P. palmivora*, the isolates from Trinidad and an isolate from Côte d'Ivoire (CIV99.2) were more aggressive than the isolates from Papua New Guinea, Ecuador, Ghana and Malaysia. One of the isolates from Nigeria (NGRpal), with very low aggressiveness, had to be deleted from the statistical analyses of assays 2 and 5. For the species *P. megakarya*, the isolates from Nigeria were more aggressive than those from Cameroon and Ghana. They were all less aggressive than the *P. palmivora* isolate TRI1. For the species *P. capsici*, the isolates from Trinidad were more aggressive than those from Malaysia and Brazil. Isolate LKM07 of the species *P. parasitica* caused very slight symptoms and was not included in the analysis.

Table 5. Classification of the isolates of the different species for their aggressiveness level measured with the leaf disc inoculation test (ordered from the least aggressive to the most aggressive; control isolates in bold)

Assay 1		Assay 2		Assay 3		Assay 4		Assay 5		Assay 6		Assay 7	
PNG1	a	NGRP	a	ASH36	a	**P517**	**a**	LKM43	a	LKM07	a	VEN1	b
PNG4	b	LKM43	b	**P517**	**a**	PNG1	a	LKM54	b	ASH36	b	BR26	b
P517	**bc**	LKM45	c	NS341	a	PNG4	a	**P517**	**c**	VEN1	b	**P517**	**c**
PNG2	cd	LKM54	c	NS269	a	CIV991	b	GH18	d	BR26	bc	**TRI1**	**d**
PNG5	cd	**TRI1**	**c**	NS331	b	**TRI1**	**b**	EQ1	e	**P517**	**c**	BR23	d
CIV99.1	cd	TRI3	c	NS328	bc	PNG3	b	TRI3	ef	BR23	cd		
PNG3	de	GH18	d	NGR20	bc	PNG5	c	PNG2	ef	NS328	d		
TRI18	ef	EQ2	d	NGR16	bc	TRI18	d	NS269	f	**TRI1**	**d**		
CIV99.2	ef	**P517**	**d**	**TRI1**	**c**	CIV992	e	NGR16	g	NGR20	de		
TRI1	**ef**	TRI19	d			TRI17	e	**TRI1**	**h**				
TRI17	f	EQ1	d										

As the means per assay were comparable and the variances were uniform, a general mean was estimated per clone and per isolate, and the coefficients of variability for the INDE7 scores were calculated. These means per clone are only indicative, since they were obtained from 2 or 3 assays only and therefore not in relation to all the isolates (Table 6). Classifications of the clone resistance levels were significantly correlated in 85% of the comparisons between isolates. The isolates for which the correlations were lowest were PNG1, NS328 and TRI3 (Table 7).

Table 6. Means (INDE7) and coefficients of variation (CV) per isolate and per clone

Isolate	Mean INDE7	CV
NGRPAL	1.06	0.29
PNG1	1.87	0.42
LKM43	1.91	0.30
VEN1	2.04	0.24
BR26	2.05	0.25
ASH36	2.08	0.23
LKM45	2.24	0.32
PNG4	2.33	0.28
LKM54	2.37	0.29
NS341	2.41	0.25
TRI3	2.46	0.32
P517	2.48	0.23
EQ2	2.51	0.29
CIV99.1	2.55	0.26
GH18	2.56	0.26
PNG2	2.60	0.20
PNG5	2.62	0.21
TRI19	2.73	0.28
BR23	2.75	0.26
NS331	2.75	0.16
NS328	2.76	0.17
NS269	2.77	0.16
PNG3	2.82	0.22
NGR16	2.88	0.18
EQ1	2.91	0.25
TRI18	2.97	0.22
TRI1	2.99	0.20
CIV99.2	3.07	0.18
NGR20	3.30	0.12
TRI17	3.34	0.18

Clone	Mean INDE7	CV
IMC47	1.58	0.35
GU255V	1.88	0.23
SCA6	1.92	0.31
PA120	1.96	0.28
AMAZ15-15	2.00	0.25
P7	2.06	0.23
GU307V	2.12	0.26
T85/799	2.23	0.33
NA33	2.26	0.26
MAN15-2	2.31	0.23
GU175V	2.58	0.24
APA4	2.62	0.21
VENC4-4	2.69	0.20
CAT1000	2.72	0.20
PA107	2.76	0.22
SNK413	2.79	0.26
IFC5	2.80	0.23
VENC22-6	2.83	0.26
T79/501	2.87	0.23
PA150	2.91	0.23
SPEC54-1	2.91	0.21
EET59	2.97	0.20
ICS1	3.00	0.20
LCTEEN46	3.02	0.25
SNK64	3.04	0.25
LAF1	3.05	0.17
MXC67	3.08	0.19
BE10	3.19	0.54
N38	3.30	0.68

Table 7. Correlations between isolates

	ASH36	CIV99.1	CIV99.2	EQ1	EQ2	GH18	LKM43	LKM45	LKM54	NGR16	NGR20	NGRPAL	NS269	NS328	NS331	NS341	P517	PNG1	PNG2	PNG3	PNG4	PNG5	TRI1	TRI17	TRI18	TRI19	TRI3
ASH36		*	*	*	*	*	*	*	*	*	*	*	*	NS	*	*	*	*	*	*			*	*	*	*	NS
CIV99.1			*	*	*	*	*	NS	*	*	*	*	*	NS	*	*	*	*	*	*	*	*	*	*	*	*	*	NS
CIV99.2				*	*	*	*	NS	*	*	*	*	*	NS	*	*	*	*	*	*	*	*	*	*	*	*	*	*
EQ1					*	*	*	*	*	*	*	*	*	NS	*	*	*	NS	*	*	*	*	*	*	*	*	*	NS
EQ2						*	*	*	*	*	*	*	*	NS	*	*	*	NS	*	*	*	*	*	*	*	*	*	NS
GH18							*	*	*	*	*	*	*	NS	*	*	*	NS	*	*	NS	NS	*	*	*	*	*	NS
LKM43								*	*	*	*	NS	*	NS	*	*	*	NS	*	*	NS	NS	*	*	*	*	*	*
LKM45									*	*	NS	NS	*	NS	NS	*	*	NS	*	*	NS	NS		*	*	*	*	NS
LKM54										*	*	*	*	NS	*	NS	*	NS	*	*	*	NS	*	*	*	*	*	NS
NGR16											*	*	*	NS	*	*	*	NS	*	*	NS	NS	*	*	*	*	*	NS
NGR20												*	*	NS	*	*	*	*	*	*	NS	NS	*	*	*	*	*	NS
NGRPAL													NS	NS	*	NS	*	NS	NS	NS	NS	NS	*	*				NS
NS269														NS	NS	NS	NS	NS	*	*	NS	NS	*	*	*	*	*	NS
NS328															NS	*	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS
NS331																NS	*	NS	*	*	NS	*	*	*	*	*	*	*
NS341																	*	*	*	*	NS	*	*	*	*	*	*	NS
P517																		NS	*	*	*	*	*	*	*	*	*	NS
PNG1																			*	*	*	*			*	*	*	NS
PNG2																				*	*	*	*	*	*	*	*	*
PNG3																					*	*	*	*	*	*	*	NS
PNG4																						NS	*	*	*	*	*	NS
PNG5																							*	*	*	*	*	NS
TRI1																								*	*	*	*	*
TRI7																									*	*	*	*
TRI18																										*	*	*
TRI19																											*	*

Discussion and conclusion

Through this study, it was possible to determine the resistance level of 32 cocoa clones widely distributed and planted in varietal trials in ten cocoa-producing countries. The 30 or so isolates of different species of *Phytophthora* used represented the major species of *Phytophthora*, hence a large share of the diversity of the pathogen in cocoa-growing regions.

The method whereby early screening is carried out on leaf discs was suited to the large number of inoculations performed (32 clones and 30 isolates). Better inoculum quality was achieved with more appropriate culture conditions, particularly the temperature level depending on the requirements of the *Phytophthora* species used. The great number of inoculations combining isolates and repetitions gives a good robustness of the results.

Classification of the clones according to infection intensity on leaves tallied with the known performance of some of those clones in the field (IMC47, SCA6 and P7). The most resistant clones were generally resistant to most of the isolates of the different species. They were IMC47, PA120, NA33, SCA6, AMAZ15-15, MAN15-2, P7, GU255V and GU307V. The most susceptible clones were LAF1, N38, EET59, VENC22-6, BE10, LCTEEN46, MXC67 and EQX3360-3. These clones particularly belonged to the Trinitario and Amelonado genetic groups, known to be generally susceptible to Ppr. A statistical analysis revealed that the isolate effects were often greater than the clone effects. The differences in aggressiveness between the isolates tested were considerable within the same species, but the average aggressiveness of the three species of *Phytophthora* was quite similar. In the species *P. palmivora*, the isolates from Trinidad and an isolate from Côte d'Ivoire were the most aggressive. For the species *P. megakarya*, the isolates from Nigeria were more aggressive than those from Cameroon and Ghana, but less aggressive than TRI1 of *P. palmivora*. For the species *P. capsici*, the isolates from Trinidad were more aggressive than those from Malaysia and Brazil. However, although there was sometimes a significant interaction effect between isolates and clones, it was weak and only very slightly modified the classification. It was therefore possible to assess cocoa tree genotypes using a single moderately aggressive isolate of *P. palmivora*, *P. capsici* or *P. megakarya*, as the results were just as valid for most of the

other isolates belonging to one of these three species. However, a few isolates gave results that were less well correlated with those of the other isolates. It is also known that the species *P. citrophthora*, which was not studied here, is highly aggressive with all the international clones, except SCA6 (Edna Luz, pers. comm.). It would therefore be worth carrying out a closer study of isolate x clone interactions with *P. citrophthora* and the most interactive isolates identified in this study.

With the results obtained at the end of that study, it will be possible to provide geneticists and plant pathologists with objective references for scales of clone susceptibility and scales of *Phytophthora* spp. isolate aggressiveness. The results obtained are highly favourable for setting up a global collaboration network for improving genetic resistance to Ppr.

Acknowledgements

We thank all the plant pathologists for sending isolates of *Phytophthora* to Montpellier: E.D. Luz and S. Silva (CEPEC/CEPLAC, Brazil), S. Nyassé (IRAD, Cameroon), I. Kébé (CNRA, Côte d'Ivoire), C. Suarez (INIAP, Ecuador), I.Y. Opoku (CRIG, Ghana), C.L. Bong (MCB, Malaysia), O.A. Olunloyo (CRIN, Nigeria), G. Blaha (CCI/CIRAD, Papua New Guinea), D. Iwaro (CRU, Trinidad and Tobago) and D. Parra (INIA, Venezuela).

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Correlations of cocoa clones' reactions to different species of *Phytophthora*

E.D.M.N. Luz[1], M.C.A. Paim[1], S.D.V.M. Silva[1], J.L. Pires[2], L.P. Santos Filho[3] and W.R. Monteiro[2]

[1] Plant Pathology Section (SEFIT); [2] Genetic Section (SEGEN); [3] Socio-Economic Section (SESOE) of CEPEC/CEPLAC, Cx. Postal 07, 45600-970, Itabuna-Bahia, Brazil

Abstract

Since more than one species of *Phytophthora* occurs in Bahia, Brazil, causing black pod disease (*Phytophthora* pod rot or Ppr), it is important to know if there will be the need to test the different clones for resistance to this disease against all three species. This paper deals with the study of the interactions of cocoa clones tested by the leaf disc test with different species of *Phytophthora* using the genotypes tested in Bahia during the activities of the CFC/ICCO/IPGRI project. It is concluded that, although some interactions were detected in resistance to different *Phytophthora* species, it seems that testing resistance to one species of *Phytophthora* may be indicative also of the resistance to other *Phytophthora* species and that resistance identified in one country could probably also be useful in other countries.

Pathogen diversity and population dynamics

Phytophthora capsici Leonian, *P. citrophthora* Leonian and *P. palmivora* (Butl.) Butler (Campelo and Luz 1981) are important pathogens of cocoa in Bahia, Brazil. These species are associated with Ppr and were responsible for severe crop losses during the 1970s and the 1980s. Although *P. heveae* Thompson has also been recorded in Bahia causing cocoa root rot (Luz *et al.* 1989), it is not considered to be a major pathogen on pods.

After the outbreak of witches' broom disease (WB) in Bahia, the incidence of Ppr decreased considerably (Luz *et al.* 2005). However, by the end of the 1990s, with the substitution of part of the traditional cocoa "Comum" plantations with genotypes more resistant to WB and the occurrence of more regular rainfall in southern Bahia, new *Phytophthora* outbreaks occurred. Interestingly, there was a gradual change in the frequency of *Phytophthora* species in the cocoa-growing area of Bahia. The *P. palmivora* and *P. citrophthora* species, which are in general more aggressive in artificial inoculation experiments than *P. capsici*, became more prevalent (Luz *et al.* 2003) than the latter species, which prevailed in the 1970s and 1980s (Campelo and Luz 1981).

The cocoa breeding programme for disease resistance in Bahia always took into consideration the need for testing cocoa genotypes against all three species, as they vary in pathogenicity to different cocoa organs (Luz 1989) and since it was found that some genotypes could react differently to one or more species (Lawrence and Luz 1982; Luz *et al.* 1996).

During the CFC/ICCO/IPGRI project in Bahia, inoculations were done, when possible, with all three *Phytophthora* species or with the two most aggressive ones (*P. palmivora* and *P. citrophthora)*. The leaf disc inoculation method (Nyassé *et al.* 1995) has been used in Bahia mainly for the early evaluation of resistance to *Phytophthora* species at the progeny level. This test has given significant correlations with field attacks (Kébé and Tahi 1999). The aim of this paper is to report on the interactions of cocoa clones tested by the leaf disc test with different species of *Phytophthora* in Bahia during the activities of this project.

Clones screened for resistance and experimental procedures

During the five years of this project, a total of 327 genotypes were tested for resistance to *P. citrophthora* and *P. palmivora* using the leaf disc assay (Nyassé *et al.* 1995). The tested genotypes included 31 "international clones", 50 clones of the local cocoa germplasm collection, 9 crosses between selected parents, 6 clones of the VB series (farmers' selection with resistance to WB) and 231 selections derived from breeding populations, named with the initials CP. Sixty-six genotypes were also inoculated with *P. capsici*, comprising 51 clones of the local cocoa germplasm collection, 9 crosses between selected parents (as above), and 6 VB clones.

A total of 14 experiments were conducted to test the 327 genotypes. The experimental conditions were the most homogeneous possible for all experiments in order to compare the results. The presence of the two control genotypes (Catongo and SCA6) in all experiments allowed the comparisons. In the first 4 experiments inoculations were done with all three species. All genotypes were inoculated at least three times, with Catongo and SCA6 included in each experiment as susceptible and resistant controls. The uniformity of the results was measured by the reactions of the controls to inoculation. SIC23, a susceptible clone according to local field observations and pod inoculations (Luz *et al.* 1996), was also included as a control in the experiments conducted after 2000. Leaves were collected in the field or in the nursery for the clones present in the International Clone Trial, Local Clone Trials and Local Clone Observation Plots. In each experiment, 5 leaf discs/clone/*Phytophthora* species were used in each of four replicates (inoculation trays), totalling 20 leaf discs for each clone x *Phytophthora* species combination for each experiment. The zoospore suspensions used in the inoculations contained 500 000 zoospores/ml. Evaluation was done 7 days after inoculation using a disease severity score varying from 0 to 5 (Nyassé *et al.* 1995).

Statistical analyses were done for each individual experiment using the average disease severity scores obtained for each species as variables in correlation analyses. In the case of experiments 1 to 4 the analyses were applied to the three pairs of species. For the other experiments, only *P. palmivora* and *P. citrophthora* were compared. Pearson's correlation coefficients were calculated using the Stepwise procedure for dependent variables of the SAS program.

Correlations among the reactions of clones to different species

There were only significant (P≤0.1) and positive correlations for the reaction of clones to *P. palmivora* and *P. citrophthora* in the first four experiments (r=0.59-0.81) in which 51 clones of the local cocoa germplasm collection were tested. In these experiments, 3 out of 4 correlations were also significant (P≤0.5) for the reactions of the clones to *P. capsici* x *P. palmivora* (r=0.51-0.66) and *P. capsici* x *P. citrophthora* (r=0.23-0.86) (Table 1).

Table 1. Pearson correlation coefficients of *Phytophthora* species interactions in four leaf disc inoculation experiments

Experiment number	*P. capsici* x *P. palmivora*	*P. capsici* x *P. citrophthora*	*P. palmivora* x *P. citrophthora*
1	0.66* (0.0017)**	0.86 (0.0001)	0.59 (0.0059)
2	0.62 (0.0026)	0.46 (0.0366)	0.81 (0.0001)
3	0.51 (0.0762)	0.75 (0.0032)	0.78 (0.0016)
4	0.63 (0.0084)	0.23 (0.3962)	0.63 (0.0093)

* Pearson correlation coefficients

** Probability

Pearson's correlation coefficients were statistically significant for 7 out of the 10 other experiments analyzed (Table 2). Values of Pearson's correlation coefficients varied from 0.01 to 0.80 when the reactions of the clones to *P. palmivora* x *P. citrophthora* were compared for these 10 experiments.

Table 2. Pearson correlation coefficients of interactions in reactions of clones to *Phytophthora palmivora* x *P. citrophthora* in ten leaf disc inoculation experiments

Experiment number	*P. palmivora* x *P. citrophthora*	Experiment number	*P. palmivora* x *P. citrophthora*
5	0.71* (0.0001)**	10	0.46 (0.0068)
6	0.79 (0.0001)	11	0.80 (0.0004)
7	0.12 (0.5214)	12	0.01 (0.9834)
8	0.58 (0.0003)	13	0.76 (0.0001)
9	0.43 (0.0129)	14	0.63 (0.0023)

***** Pearson correlation coefficients
** Probability

Van der Vossen (1997) stated that the ranking order for clones tested for resistance to Ppr in Cameroon or Togo against *P. megakarya* was very similar to that for Ppr caused by *P. palmivora* in Côte d'Ivoire. In Trinidad, Iwaro *et al.* (1998) also reported a similarity in the ranking of clones inoculated with *P. palmivora* and *P. capsici*. The results found in the present study support the previous results of Van der Vossen (1997) and Iwaro *et al.* (1998), although for some experiments it was possible to observe some degree of differential reactions of the clones to more than one species (Table 3), as previously stated by Lawrence and Luz (1982) and Luz *et al.* (1996). Clones often show a resistant reaction to *P. capsici* or *P. palmivora* and an intermediate or low resistance reaction to *P. citrophthora*, which is in general the most aggressive species. This means that, although different genes may be associated with resistance to different *Phytophthora* species, they are strongly linked. Based on these observations the results obtained when testing resistance to one species of *Phytophthora* may be of value to other species and similarly from country to country, contradicting the recommendation of Lawrence (1983).

Table 3. Example of the degree of differential reactions that can be observed for disease severity of some clones inoculated in the same experiment with three *Phytophthora* species

Clone	Average of *disease* severity scores		
	P. capsici	*P. palmivora*	*P. citrophthora*
BO8	0.95	1.98	4.17
CEPEC74	0.95	1.83	3.00
CSUL9	1.87	2.07	3.38
NA33	1.07	2.55	3.08
Pound11	1.26	3.53	3.63
SIC23	0.52	1.80	2.50

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WITCHES' BROOM RESISTANCE STUDIES

Review of resistance screening of cocoa seedlings and clones to witches' broom disease: methods, problems and correlation with field results. The experience of the Cocoa Research Unit, Trinidad

J.-M. Thévenin[1], R. Umaharan[2] and D.R. Butler[2]

[1] CIRAD-CP, TA 80/02, Avenue Agropolis, 34398 Montpellier cedex 5, France

[2] Cocoa Research Unit (CRU), The University of the West Indies, St. Augustine, Trinidad and Tobago

Abstract

Developing screening tests to assess the level of resistance of a perennial crop such as cocoa towards diseases is essential to reduce the length of the breeding cycle. In the case of witches' broom disease caused by *Crinipellis perniciosa*, major constraints are linked to the fact that symptoms develop only when meristematic tissues are attacked, to the time needed for symptoms to develop, and to the low correlation between levels of attacks on flower cushion, twigs and cherelles. Field assessment under natural conditions of infection can produce valuable information but the status of clones showing some resistance in the field should ideally be confirmed using an inoculation method in the nursery, especially when the inoculum pressure in the field is low. The experience obtained at the Cocoa Research Unit (CRU) with field observations and with artificial inoculations and other resistance tests over the past decade is described. Progress with artificial inoculation tests has been significant, but the development of a reliable *in vitro* test, including germination of spores in plant extracts, remains difficult due to the complexity of the *Theobroma cacao-Crinipellis perniciosa* pathosystem.

Introduction

Crinipellis perniciosa (recently renamed *Moniliophthora perniciosa),* a basidiomycete fungus causing witches' broom (WB) disease on cocoa, is a pathogen infecting meristematic tissues: active flower cushions, cherelles and active vegetative buds. It is not uncommon for some clones to be resistant to one form of the disease and susceptible to another form of the disease. Pires *et al.* (1999) and Thévenin *et al.* (2003) reported relatively low but significant coefficients of correlation between the levels of attack on twigs, pods and flower cushions.

The level of resistance of cocoa to WB can be evaluated in the field under natural conditions of infection. Although this method has the advantage of assessing the resistance of the plant in its environment, for the observations to be robust, large plots with statistical experimental designs are required. Observations are time-consuming as several years are needed for the establishment of the plants and of the disease in the plots, especially when observations on pods need to be made.

To reduce the length of the breeding cycle, it is necessary to develop tests that can be performed on young plants in the nursery or in the laboratory for the evaluation of the resistance on vegetative parts. But it is also necessary to develop tests to assess the level of resistance on pods. Numerous attempts have been made to develop methods to assess the resistance of cocoa to WB. Most of them depend on using the vegetative parts of live plants: inoculation of germinating seeds, of young seedlings and of clonal material. Attempts have also been made to develop tests less dependent on the environmental conditions: inoculation of detached leaves and of callus tissue, or methods based on the germination of basidiospores in plant extracts. Most of the methods gave at one point in time promising results but failed to be fully reliable, subsequently recording inconsistencies in the results.

At the time when the CFC/ICCO/IPGRI project *"Cocoa Germplasm Utilization and Conservation: a Global Approach"* and the ACRI/WCF project *"Evaluation of Cocoa Germplasm for Resistance to Witches' Broom Disease"* were initiated in Trinidad, the most popular screening method was that developed by Purdy *et al.* (1997). This method became the basis for the evaluation of cocoa germplasm in controlled conditions in Trinidad from 1998, however field observations were also conducted in the International Cocoa Genebank, Trinidad (ICG,T). Simultaneously, alternative methods were under development.

Field assessment for witches' broom resistance

Methodology

Field observations were conducted over a 3-year period (November 1998 to December 2001) in the ICG,T. A total of 475 clones were assessed: 61 clones for 3 years, 101 clones for 2 years and 313 for 1 year only. Depending on the availability of trees for each clone, one to five trees were selected to assess symptoms of black pod (*Phytophthora* pod rot or Ppr) on pods and symptoms of WB both on pods and shoots.

For observations on pods, each tree was observed monthly; ripe pods were harvested and the number of healthy pods and the number of pods with WB and/or Ppr symptoms were recorded. For observations on shoots, three branches of approximately 1.5 m in length were selected to represent the canopy from each selected tree. On each branch, records were taken of the number of healthy shoots and the number of brooms and these brooms were tagged. The observations on shoots were made three times per year, in November, March and July.

Results

For the 61 clones observed for 3 years, linear correlation between years was highly significant, with coefficients of correlation varying between 0.51 and 0.74 for Ppr, between 0.39 and 0.64 for WB on pods and between 0.77 and 0.89 for WB on shoots. Correlation between traits was also highly significant: the Spearman coefficient of correlation between Ppr and WB on pods was estimated at 0.31 (phenotypic correlation) and 0.32 (genotypic correlation), whereas it was estimated at 0.29 (phenotypic correlation) and 0.45 (genotypic correlation) between WB on pods and WB on shoots.

The global percentage of pods affected by Ppr over the period of observation was estimated at 24.3% for the 354 clones kept for the analysis; 25.7% of the accessions were considered to be resistant (less than 10% of pods affected), 36.4% to be moderately resistant (between 10 and 25%) and 37.9 % to be susceptible (more than 25% of pods affected). All populations (groups of accessions sharing the same acronym) considered in this study had clones in each of the resistance classes**.**

The global percentage of pods affected by WB was estimated at 6.8% for the same 354 clones analyzed; 57.9% of the accessions had less than 5% of their pods affected by WB, 16.1% had 5-10% of their pods attacked and the remaining 26% had more than 10% of pods affected by WB.

The global percentage of shoots affected by WB was estimated at 0.47% for the 472 clones analyzed. A large percentage of clones (39.2%) had no brooms or less than 0.01% of their shoots affected by WB and could be considered to be very resistant to the disease. Another large group (38.6%) had 0.01 to 0.5 % of their shoots affected by WB and could be considered to be resistant, whereas 17.2% of the clones had a rate of attack of their shoots between 0.5 and 2.5%. Five percent of the clones observed were considered to be very susceptible with more than 5% of shoots affected by WB.

There could be several possible reasons for the high percentage of "resistant" clones: (i) most of the clones were initially selected as seed from mother trees with apparent resistance to WB (Pound's expeditions) and, therefore, there is a good representation of WB resistance in the ICG,T; (ii) the pathotype ("B") of *C. perniciosa* present in Trinidad is less aggressive than pathotype "A" prevalent on the western side of the Andes, and would therefore induce less symptoms; hence, the percentages of infection will seem small even for susceptible clones.

Problems encountered and limitations

The possible environmental heterogeneity within a field combined with differences of weather patterns from one year to another mean that field observations on pests and diseases of a perennial crop such as cocoa need to use a suitable experimental design and for several years. Genebanks (and cocoa plantations in general) take a number of years to establish and bear fruits. Diseases and pests affecting this crop will also need a few years to establish, depending on the target organ for attack. Most cocoa-producing countries have their own genebank, which is very often not planted with an experimental design which would allow reliable statistical analysis of field observations.

Reliable, quick screening tests are however not always available to evaluate the level of resistance to certain pests or diseases under controlled conditions. It therefore becomes necessary to take full advantage of established genebanks by conducting field observations on diseases and pests present in the respective countries and to provide an idea of the level of resistance of living genetic material in the local environment.

Another advantage of field observations is that several traits can be assessed at one time (e.g. damage on pods from several diseases and pests in one round of harvest). In a well established cocoa plantation where WB pressure is also well established, observations on WB attacks on shoots can be facilitated by counting only the number of brooms, instead of both healthy and affected shoots. This is possible since the number of brooms was found to be highly and significantly correlated to the percentage of shoots affected by WB. Field observations in the ICG,T generate useful data for the selection of genotypes for further use in breeding programmes. A quick negative screening for the level of WB infection on shoots would eliminate clones susceptible to this form of WB disease. Observations on pods on the remaining accessions would permit the detection of potential sources of resistance that would need to be confirmed using other methods.

Promising resistant accessions

Among 162 accessions observed for at least 2 years, 27 clones were selected, based on the following traits: trees having produced at least 10 pods per year, and with the percentage of pods affected by Ppr less than 10%, the percentage of pods affected by WB less than 5% and the percentage of shoots affected by WB less than 0.05%. These are:

AM1/73 [POU]	MOQ6/99	PA195 [PER]
B12/1 [POU]	NA70	PA296 [PER]
CRU101	NA289	PA303 [PER]
CRU104	NA471	Playa Alta 2
CRUZ7/8	NA669	RB29 [BRA]
GU241/P	NA680	RIM13 [MEX]
IMC47	PA32 [PER]	SLC4
JA3/4 [POU]	PA120 [PER]	SPA9
MAN15/60 [BRA]	PA171 [PER]	SPEC194/103

Screening for resistance to witches' broom disease in the nursery

Methodology

A project funded by the American Cocoa Research Institute (ACRI) and subsequently by the World Cocoa Foundation (WCF) was initiated in 1998 with the main objective of screening accessions of the ICG,T under controlled conditions for resistance to WB. The level of resistance of clones showing promise from mass screening by spray inoculation would then be confirmed and quantified.

The method recommended by ACRI for the mass screening exercise was that developed by Purdy *et al.* (1997) at the University of Florida on seedlings, using an automated spraying system. However, a number of modifications had to be made to the methodology in order to adapt it to the conditions of Trinidad (see "Problems encountered and limitations" below). Inoculations were carried out by spraying, either with a modified version of the overhead spray system (air/liquid atomizing nozzles) or manually using a Preval Sprayer (Precision Valve Corp., NY, USA), which delivers a fine spray equivalent to the automated overhead spray system. Prior to inoculation, plants were wetted and kept in an incubation chamber for 24 hours under controlled conditions (25°C, humidity close to saturation, darkness). Approximately 1 ml of inoculum at a concentration of 350 000 basidiospores/ml was delivered to each plant. Plants were then left again in the incubation room for 60 hours to facilitate germination of the basidiospores and the infection process of the cocoa tissue by the fungus.

Grafted plants on homogeneous TSH rootstocks were inoculated. Prior to inoculation, plants were pruned to induce synchronized flushing. Plants were inoculated at the bud-breaking stage. Following incubation, plants were kept in a shaded nursery, where symptoms were observed for a 4-month period. A rapid qualitative assessment was first done to assess the type of symptom (swelling of petiole, of stem or shoot, canker, green or dry broom). A quantitative assessment followed, with measurements of the severity of the symptoms (diameter of swellings and characteristics of the brooms).

Results

By December 2003, a total of 22 000 TSH rootstocks were established for grafting, and 883 accessions from the ICG,T were grafted. During each grafting exercise, 750 plants were propagated (15 replicates of each of the 50 accessions selected for each series). Overall, 706 accessions were inoculated. The evaluation was completed for 553 of these accessions, belonging to 53 accession groups.

The correlation between the first 5 inoculation series for 13 control clones varied from 0.18 to 0.87 for the Pearson coefficient of correlation and from 0.10 to 1 for the Spearman coefficient of correlation, and was not always significant. On average over the same series, the Spearman coefficient of correlation between the percentage of shoots affected in the nursery and the percentage of shoots affected in the field for eight control clones for which field data were available was 0.73 ($P=0.037$). This underscores the variation that can be observed between similar inoculation series and demonstrates the need for adequate replication.

Overall, 32% of the clones showed less than 20% of infection, and IMC was the accession group having the highest percentage of resistant accessions (58.8%). Within the Upper Amazon Forastero (UAF) and Refractario groups, approximately 33% of the accessions had less than 20% of infection, whereas similar levels of infection were found in only 24% of Trinitario accessions and 11% of Lower Amazon Forastero (LAF) accessions.

Measurements of broom-base diameter and broom length were shown to vary significantly among clones ($P<0.01$), but there was no significant correlation between the percentage of infection and the broom-base diameter, both observed in the nursery.

The linear and rank correlations between 3 years' field assessments and screening experiments in the nursery were not significant for the percentage of infection (considering all types of symptoms or brooms only) when individual tree data were considered (i.e. when the same trees were used for field observation as were used for the budwood for grafting in the nursery). A slightly significant rank correlation (r=0.13, P=0.056) was found with a clone-by-clone comparison (taking the average of all replicated trees in the field and the average of all grafted plants for each clone). The coefficient of correlation became more significant (r=0.89, P=0.018) when the percentage of shoots developing brooms was considered and when six classes of resistance based on the field observations (instead of individual clones) were correlated. We therefore concluded that the nursery inoculation method and the field assessment method allowed classification into rough classes of resistance, but that the individual level of resistance of each clone (and especially the confirmation of the resistant clones) would need to be quantified using another method.

Selection of promising clones for confirmation

A two-tiered screening system seemed the most suitable screening protocol for WB resistance. Clones were selected to be tested again for confirmation of their resistance when results were available for at least 3 replicates (grafted plants) with 4 shoots for each replicate. Plants were identified as "putatively resistant" based on the percentage of infection (less than 20% infection or no brooms) and on the basis of broom development (broom-base diameter less than that of the most resistant control or less than 6 mm at maturity).

Using these criteria, 111 accessions were selected for further confirmation of their resistance. They belong to various populations (Table 1).

Table 1. Populations with promising accessions from the results of screening by spray inoculation. Putatively resistant accessions were selected according to the criteria given in the text.

Population	No. of accessions	Accessions
AM	3	2/62, 2/87, 2/92
AMAZ	2	3/2, 12
B	9	1/2-24, 2/16, 9/10-25, 9/10-30, 13/5, 14/13, 18/4, 23/1, 23/2
CC	1	10
CL	4	10/15, 13/4, 13/65, 19/10
CLM	1	91
CRU	6	19, 35, 52, 56, 87, 101
DOM	3	18, 21, 24
EET	5	19, 58, 162, 272, 397, 400
GS	1	26
GU	1	195
ICS	8	4, 8, 16, 25, 30, 43, 45, 46
IMC	10	14, 16, 36, 38, 45, 55, 58, 63, 76, 78
JA	1	1/21
LCTEEN	1	31
LP	8	1/24, 3/4, 3/15, 4/8, 4/18, 4/20, 4/24, 4/52
LV	3	17, 20, 28
Matina	1	1/7
MO	1	9
MOQ	6	1/21, 3/8, 4/20, 4/21, 6/46, 6/52
NA	6	70, 137, 159, 170, 268, 669
PA	11	30, 34, 67, 70, 71, 125, 126, 169, 279, 289, 303
Playa Alta	1	2
Pound	4	2/A, 4/A, 10/B, 16/B
SCA	2	2, 3
SJ	3	1/19, 2/19, 2/25
SLA	2	8, 44
SLC	2	4, 19
SPEC	1	184/2
UF	3	29, 168, 667

Among these accessions, 35 were found resistant in the field, 18 moderately resistant, 10 susceptible and 48 had an unknown infection level in the field.

Problems encountered and limitations

The method of inoculation developed in Florida was designed for use with seedlings, whereas the screening at CRU was conducted on clonal material. The propagation of plants was not always an easy task and, on many occasions, we had to face dieback of a large number of the scions within a few weeks of the first emergence of young flushes. There was no clear reason why this occurred although attacks by opportunistic fungi were suspected. To be well organized for inoculations it was necessary to synchronize the production of flushes, but this was not easy as the timing was clone-dependent. It was necessary to manipulate the plants by heavy pruning, fertilization and watering to ensure more synchronous flushing.

New inoculum storage facilities and new broom cabinets (for production of basidiocarps) were needed to provide the large quantity of inoculum required. The heavy weight of grafted plants, the heterogeneity in plant height and a lack of space at CRU made it difficult to adopt the belt system used in Florida for the clonal plants. Instead, it was decided to use a mobile overhead spray unit that would be adjustable in height. This system was used for several inoculation series. However, it was found that a significant amount of inoculum was evaporating in the air during the inoculation process and that some cocoa leaves dried during their transfer to the incubation chamber in the adjacent room. This could explain some of the inconsistent results among the early series of inoculations. Subsequently it was decided to inoculate plants with a hand atomizer directly in the incubation chamber where conditions for the infection process were ideal.

Designing an adequate system to ensure controlled temperature and high relative humidity in the incubation chamber throughout the year was also not straightforward. High temperatures that prevail during the dry season required the use of an air-conditioning unit, since the use of drapes wetted continuously with running water (as used in Florida) did not cool the air adequately and would have consumed too much water. However, the air conditioner also desiccated the air, so it was necessary to increase the humidity in the chamber. After experimenting with several systems, the best conditions were obtained with a large walk-in plastic chamber inside the incubation room containing water baths with thermostats to generate steam inside the plastic chamber, the air-conditioning unit being outside the plastic chamber.

In conclusion, the methodology used allowed many plants to be screened simultaneously, and permitted the differentiation between clones with contrasting levels of resistance, provided that enough replicates were used. However, for each experiment, the time frame from the raising of rootstocks and the propagation of the accessions to the "final" results was around 14-16 months. Furthermore, despite all precautions taken, some experiments failed and several sets of results were inconsistent. The need for another method to confirm and quantify the level of resistance of putatively resistant clones from the initial screening exercise is therefore essential. In addition, an alternative method less dependent on the environment which could also be used as an early screening test in pre-breeding and breeding programmes is needed.

Alternative methods

As noted above, none of the methods developed and used so far proved to be fully reliable for evaluation of clonal material. Efforts were made to develop or test a broader spectrum of methods that could be used independently of the kind of plant material to be tested (clones or seedlings).

Assessing the level of resistance of clones through their progenies

Clones producing pods may be assessed for their level of resistance to WB through the inoculation of seedlings, obtained from open- or hand-pollinated pods, providing that a strong heritability exists. This type of test is not destructive for the clones themselves and can therefore be repeated as many times as needed. Inoculation of very young seedlings (1-week-old) was performed by Holliday (1955) and Bartley (1959) by dipping the germinated seeds from open-pollinated pods into a suspension of basidiospores. Others have used spray inoculations of older seedlings (e.g. the belt spray method) or droplet inoculations (Surujdeo-Maharaj *et al*. 2004).

We saw earlier that inoculation of seedlings or clonal material using the spray method and measuring the percentage of plants (or buds) infected can produce inconsistent results. As a recommended method for the CFC/ICCO/IPGRI project, Ducamp and Thévenin (2000) proposed the inoculation of buds using a drop of inoculum in agar. This method was first described by Sreenivasan (1987) and was subsequently used in studies on cocoa seedlings (Dabydeen and Sreenivasan 1989). Surujdeo-Maharaj *et al*. (2003, 2004) modified and developed this method with the aim of obtaining very high infection levels. They concluded that resistance of cocoa was best evaluated by measuring the incubation period (time from the inoculation to the appearance of the first symptom) and the severity of symptoms (e.g. broom-base diameter). One of the major differences between this and other existing inoculation methods is that symptoms are produced even on resistant clones, which allows symptom severity to be estimated.

This type of test must however be further improved when it comes to evaluating the level of resistance of individual young seedlings for which no replication exists. By inducing symptoms on supposedly resistant material, there is a chance of losing this valuable material. Since most of the susceptible material is killed as well, there is no way to validate the method by planting susceptible and resistant material in the field for assessment under natural conditions of infection. Following the results of Surujdeo-Maharaj *et al*. (2003, 2004), several additional experiments were set up in order to investigate whether this method could be used on young seedlings (4-16 weeks) in a pre-breeding programme. It was concluded that 8-week-old seedlings, not pruned and inoculated with a 300 000 basidiospores/ml suspension in agar, could be the basis of a test on young seedlings. Repeatability of the test and the possible development of acquired resistance due to successive inoculations of the same seedlings (to achieve replicated inoculations) have however to be further studied.

Laboratory tests for direct evaluation of clonal material

Inoculation of callus tissues (Evans 1980; Fonseca and Wheeler 1990; Muse *et al*. 1996) could be used to assess the level of resistance of adult plants or newly created progenies. It is a non-destructive test, which is however costly and for which much developmental work is needed to standardize the method, especially in terms of the assessment of symptoms.

Inoculation of detached leaves was tried by Ducamp (1995). The method involved inoculating cocoa leaves with drops of basidiospores suspensions containing 1-2% glycerol. Symptoms observed and measured were intended to be the result of the toxicity of glycerol conveyed by the mycelium of *C. perniciosa* growing into the leaf tissue. Such a test would have been ideal as it is non-destructive, cheap and fast and can be used for adult plants as well as for seedlings in the nursery. However, standardization of this test appeared to be difficult. An alternative (though less simple) approach could be to clear leaves after infection, stain the mycelium and measure its development under the microscope.

The germination of basidiospores in phloem sap as described by Bastos and Albuquerque (2000) would also be an interesting test to evaluate the resistance of adult plants. It is cheap and fast but cannot be used on young trees in the field and even less on seedlings, due to the

wound needed to collect sap. Problems of repeatability were mentioned during the current workshop by researchers from CEPEC (Centro de Pesquisas do Cacau, Brazil).

Many experiments were conducted at CRU on germination of basidiospores in plant extracts, following the results obtained by Evans and Bastos (1980) and Brownlee *et al.* (1990a, b). Several methods were tested, varying the type of plant material (active/dormant buds, leaves from several ages), the type of solvent, the temperature of extraction, the preparation of samples, etc. The percentage of germinated spores and the length and width of germtubes were assessed four hours after the contact between spores and the extracts. This method gave promising results: the percentage of germination in extracts from susceptible clones was equivalent to the percentage in water, and generally much higher than in extracts from resistant clones. Furthermore, the length of the germtubes was shorter in extracts from resistant clones as compared to susceptible clones. The extracts gave stable results over a 3-week period when stored in the freezer at a temperature of -20°C. However, many inconsistencies were also recorded and it has not been possible, despite all efforts made, to standardize the method.

Conclusion

It has been, and probably will be for some time in the future, rather difficult to develop an *in vitro* screening test or a test based on germination of spores in plant extracts. This is apparently due to the complexity of the *Theobroma cacao–Crinipellis perniciosa* pathosystem. If such a test were independent of environmental factors, quick, reliable, cheap and applicable to young plants, it would be an ideal tool to measure the intrinsic resistance of cocoa, especially in large breeding or pre-breeding programmes. For the time being, and despite their inconvenience, observations in the field and tests based on plant inoculations provide the most useful and relevant information on cocoa resistance to witches' broom, so long as enough replications are used. Nevertheless, our results suggest the need to confirm information obtained by one method using an alternative method.

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Resistance to witches' broom disease of cocoa in Brazil: methods of inoculation, problems and correlations with field results

E.D.M.N. Luz, S.D.V.M. Silva and M.C.A. Paim
CEPEC/CEPLAC, CP 07, 45600-970, Itabuna, Bahia, Brazil

Abstract
Germplasm enhancement for resistance to witches' broom disease was initiated in 1993 as an activity in the CEPEC/CEPLAC cocoa breeding programme. The main objectives of this programme are to develop cocoa cultivars and populations with enhanced resistance to witches' broom, while maintaining a broad genetic base and high productivity. Results of the belt spray inoculation method, used routinely to test hand-pollinated crosses and seedling progenies obtained by open-pollination from selected clonal accessions, are presented. The belt spray results were significantly correlated with two variables of field resistance observed over a 5-year period (number of healthy plants and average number of brooms per plant), although the linear coefficients of correlation were relatively low. The belt spray inoculation of rooted cuttings resulted so far in low infection levels. Attempts to use leaf disc inoculations and germination of basidiospores in stem sap and apoplastic leaf fluids are also reported. The problems faced with the use of these alternative methods are highlighted. The need for further studies to improve the inoculation methods to evaluate resistance of cocoa genotypes to witches' broom is recognized.

Introduction
The development of cocoa genotypes with resistance to witches' broom (WB), caused by *Crinipellis perniciosa* (recently renamed *Moniliophthora perniciosa*) appears to be the only feasible long-term solution for the control of this serious disease of *Theobroma cacao* L. The germplasm collection of the Centro de Pesquisas do Cacau/Comissão Executiva do Plano da Lavoura Cacaueira (CEPEC/CEPLAC), rich in sources of resistance to major diseases and superior agronomic traits, remains partially unexplored. A major objective of the CEPEC cocoa breeding programme is to develop new clones and other types of cultivars presenting both disease resistance and high productivity, exploiting as much as possible the diversity available in the collection.

Evaluation of seedlings from open-pollinated and hand-pollinated progenies for WB resistance was initiated in Bahia, Brazil since 1993 using the belt spray inoculation method applied to seedling populations (Frias 1987; Luz *et al*. 2000). However an early, reliable, repeatable, fast and easy method for screening clonal genotypes for WB resistance was lacking. The objectives of the work reported here were: i) development of methods to evaluate the level of resistance of clonal cocoa material; ii) rapid assessment of disease resistance of cocoa genotypes used in the breeding programme; iii) study of the correlation of results obtained with the belt spray method with field disease assessment.

Alternative methods to assess resistance of clonal tissue

Leaf disc inoculations
The methodology used was similar to that developed to test cocoa genotypes for black pod (*Phytophthora* pod rot or Ppr) resistance (Nyassé *et al.* 1995). The inoculum concentration used was 10^5 basidiospores/ml with 3% of glycerol. The method was based on the idea that the

glycerol is introduced into the leaf with the penetrating fungal hyphae. This would cause some toxicity symptoms in young cocoa leaves only if germination and penetration is abundant. However, despite a large amount of testing, no visible infections were observed until 10 days after inoculation and the leaf discs became dry and oxidized afterwards. Therefore this method was abandoned.

Leaf apoplastic fluid

Many attempts were carried out to obtain apoplastic fluid of cocoa leaves following the methods described by Rathmell and Siqueira (1974), Machado (1991), Braren *et al.* (1994) and Bestwick *et al.* (1988). The inoculum concentration used was 10^5 basidiospores/ml in a proportion of 1:1 with the apoplastic fluid of each clone. The percentage of germination was determined after 4 hours. The main difficulties were the scant amount of fluid obtained even from a large number of leaves, due to the lack of a refrigerated centrifuge for fluid extraction, and the available centrifuge tubes capacity. However, it was possible to observe that in the four tests done the average percentage of germination of basidiospores of *C. perniciosa* in the apoplastic fluid of the resistant SCA6 clone was significantly lower (3%) than in the fluid of the susceptible SIC23 clone (45%). We consider that it might be worth continuing to develop this method under more suitable centrifugation conditions.

Stem sap test

Basidiospores were germinated in cocoa xylem and/or phloem sap of resistant and susceptible cocoa clones according to the methodology described by Bastos and Albuquerque (2000), using inoculum concentrations of 10^4, 10^5 and 10^6 basidiospores/ml. These tests were repeated several times in our laboratory in Bahia, but the results were not consistent between tests. The methodology was considered unreliable although in some of the more successful trials it was possible to separate the resistant and susceptible genotypes. Even if the technique is improved to obtain more consistent results, two major constraints were observed: i) the holes made in plants to get the sap can become infection points for *Ceratocystis* wilt, which has become an important new disease in cocoa plantations in Bahia, and ii) the relatively large efforts and time necessary to be able to improve the methodology.

Use of the belt spray method

Methods and results

The cocoa breeding programme in Bahia has been using this method since 1993 mainly to test seedling progenies obtained by open-pollination and cross-progenies obtained by hand-pollination. This evaluation programme was sponsored by the American Cocoa Research Institute (ACRI, now the World Cocoa Foundation, WCF). A total of 341 open-pollinated progenies and 189 hand-pollinated crosses (112 plants/cross) were tested using the methodology described by Frias (1987), with adaptations made to fit the local CEPEC conditions (Luz *et al.* 2000; Silva *et al.* 2001). Data were collected 60 days after inoculation, as described in the previously cited publications, and the variable considered for correlation with field results was the value of the disease index (DI) calculated for each seedling progeny by the following equation:

$$(DI = TB+AB+CB)/112$$

Where:

TB = number of plants among the 112 plants inoculated that presented terminal brooms

AB = number of plants among the 112 plants inoculated that presented axillary brooms

CB = number of plants among the 112 plants inoculated that presented cotyledonary brooms

A single progeny may present plants with all three types of infection and also plants with only one or two of the above-mentioned symptoms, taken into account in the disease index.

A sample of 100 seedling progenies evaluated through the belt system inoculation method using 7.5 x 10^4 basidiospores/ml was chosen for correlation studies with field disease incidence. Thirty-six plants from each of the 100 seedling progenies that did not show symptoms of WB after the belt spray inoculation were transplanted to the field under adult cocoa trees that were severely infected with *C. perniciosa* (Luz *et al*. 2001). The number of diseased plants, the number of brooms per plant, the number of months until first symptom appearance and the number of healthy and infected pods per plant were assessed monthly.

Data over all years of field observations were used to calculate for each progeny the following variables:

HP = total final number of healthy plants amongst the 36 plants of each progeny, at the end of the observation period

ATFS = average number of months to observe first symptoms

ATBP = average number of months to first broom development

ANB = average number of brooms per tree (for all 36 plants of each progeny)

Pearson's coefficients of correlation were calculated between each variable and the DI obtained with the belt spray method using the stepwise procedure for dependent variables of the SAS statistical programme. Table 1 shows the correlation coefficients and the F values obtained for each variable.

Although the values of the correlation coefficients were relatively low, they were significant for the variables HP and ATBP ($P \leq 0.01$). The most promising progenies for DI, HP and ATFS are listed in Table 2.

Other variables such as the percentage of infected pods and of infected flower cushions per plant, once these data become available, will be analyzed for each progeny and in each field experiment.

Table 1. Pearson correlation coefficients and level of significance (P values) for 100 progenies tested for witches' broom resistance with the belt spray method and under field conditions (for explanation of field variables see text)

Belt spray method		Field variables			
		HP	ATFS	ATBP	ANB
Disease index (DI)	r =	-0.33	0.11	0.15	-0.03
	P =	0.001	0.0585	0.0078	0.6172

Table 2. Parental clones of open-pollinated seedling progenies and hand-pollinated crosses with good levels of resistance to WB in field and greenhouse evaluations

Promising clones					Promising crosses
CAB0153	CAB0992	CEPEC92	VB000	VB548	CEPEC86 x SCA6
CAB0214	CAB153	CEPEC94	VB190	VB554	NA33 x RB39
CAB0219	CAB195	EET45	VB226	VB676	RB36 x SCA12
CAB0221	CAB269	EET59	VB231	VB681	RB36 x SCA6
CAB0233	CAB274	SCA12	VB424	VB902	SIAL70 x SCA12
CAB0329	CAB5336/19	SCA6	VB430		"Theobahia" (SCA6 x ICS1)
CAB0334	CEPEC74	TSA644	VB472		
CAB0356	CEPEC84	TSA654	VB483		
CAB0383	CEPEC90	TSH516	VB515		

Problems faced in comparing laboratory and field results

As demonstrated previously (Silva 1997; Luz *et al.* 1999), an extremely high variation in symptom expression was obtained even for plants from the same open-pollinated seedling progeny after inoculation using the belt spray method. This may largely be due to the heterozygosity of the parent(s) involved.

To correlate the results of greenhouse inoculations with natural infection in the field, it would also be best to use the same number of non-inoculated plants in both conditions, and to use replicated field plots. With the current data, 112 seedlings of open-pollinated progenies were tested under greenhouse conditions while only 36 plants were sometimes evaluated in different field plots and in different years. With this experimental procedure we consider that it would be possible to obtain only a rough idea about the relationship between the results obtained in the field and in the greenhouse.

The possible problems in the experimental design might be corrected in ongoing trials with hand-pollinated crosses. In these trials 56 plants of each cross were used both for belt spray inoculation and for establishment in field plots. The plants that did not show symptoms after the screening procedure were also planted in the field. We expect that the results from these trials will give us a more accurate idea about the relationship between the belt spray inoculation method and field resistance.

Inoculation of clones using the belt spray and agar droplet methods

One hundred and sixty-four clones used as parents of the progenies evaluated in the screening programme for WB resistance were multiplied as rooted cuttings to be evaluated for resistance during the project. The rooted cuttings were inoculated at the second flushing stage using the belt spray system with an inoculum concentration of 10^5 basidiospores/ml. In general, only a low number of the inoculated plants showed symptoms. Even rooted cuttings of the more susceptible clones according to field observations did not present high levels of infection.

The following problems may explain the low infection success: a) irregular flushing, b) difficulties in obtaining equal number of plants of each clone to be inoculated simultaneously, c) difficulties in obtaining branches for rooting of cuttings from different clones at an adequate development stage.

In view of the problems of infecting rooted cuttings using the belt spray system, the agar drop inoculation method was tried on a limited number of plants (15 rooted cuttings of each of 25 clones). The same inoculum concentration as applied for the belt spray method was used. The number of infected plants of Catongo was still low (35%), as compared to that observed for open-pollinated progenies of this clone inoculated with the belt spray method (60-80%). More work should be done to improve the methods to evaluate resistance of clones to witches' broom in Bahia.

Conclusions and perspectives

So far, the population breeding programme for WB resistance has progressed satisfactorily over the last years. Based on the results obtained until now, one can be optimistic that further improvement could be achieved as the evaluation exercise progresses and more promising genotypes combining good yield potential with resistance to WB are likely to be identified. The promising genotypes selected were used as parents of crosses for the second cycle of the population breeding programme. In addition, some of the selected clones were transferred to the "Biofabrica" (facility for mass multiplication of WB-resistant cocoa varieties in Bahia) for distribution as promising resistant varieties to interested farmers.

The high levels of WB pod infection in the field and the observation of differential responses of genotypes to shoot and pod infection has led to the need of developing also a screening method to test the level of resistance of pods from different genotypes. Clones with high and low percentage of pods infected by WB will be used to test the methodology that is still under development.

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Witches' broom resistance screening of seedlings and clones in Ecuador: some comparisons between natural infection in the field and artificial inoculations

C. Suarez-Capello[1], R. Delgado[1], P. del Pozo[1], A. Vasco[1], J. Zambrano[1], A.B. Eskes[2] and F.M. Amores[1]

[1] INIAP, EETP, Km 5, 5 Vía Quevedo-Empalme, Quevedo, Los Ríos, Ecuador

[2] IPGRI/CIRAD, c/o INIBAP, Parc Scientifique Agropolis II, 34397 Montpellier cedex 5, France

Abstract

The search for resistance against witches' broom caused by *Crinipellis perniciosa* is still handicapped by the complexity of the pathosystem and the lack of studies that establish a reliable relationship between artificial inoculation in the laboratory or nursery with natural infection in the field. The CFC/ICCO/IPGRI project has provided opportunities to test a large number of accessions under different circumstances and with different methodologies. The results herein reported, though still considered as preliminary, show some of the promises and constraints encountered with field observations and artificial inoculation of clones and seedlings in Ecuador.

In the first study, the levels of natural infection of the accessions of the International Clone Trial (ICT) in the budwood garden and in the ICT were compared. Artificial inoculations of the same clones were carried out in the shade house with the belt spray method and in the budwood garden by using agar blocks. The natural infection in the budwood garden was correlated with the results in the ICT, suggesting that natural infection of a relatively low number of trees can already give quite reliable results. The most resistant clones appeared to be SCA24, LCTEEN46, MXC67, EQX3360-3, GU255V, CCN51, PA107, SCA6 and IMC47. The results of artificial inoculations showed low infection levels of the clones both in the shade house and in the budwood garden, and there was no clear relationship with field results. In the budwood garden and sometimes also in the shade house inoculated shoots became necrotic some time after inoculation. Improvement of the conditions for inoculation of clonal plants is necessary.

In the second study, 14 seedling progenies were inoculated twice with the belt spray method. A group of 139 symptomless seedlings was established in the field besides an equal number of seedlings from the same progenies that had not been inoculated. The average number of brooms per tree observed in the field, when corrected for plant size, was correlated (r=0.57) with the percentage of infection of the progenies obtained with the belt spray method. However, the number of brooms for symptomless inoculated seedlings and non-inoculated seedlings did not show any difference. This suggests that the belt spray method is possibly efficient for estimating the average level of field resistance of seedling progenies but not so for selection of more resistant individual seedlings within seedling progenies.

The above results are discussed in the context of the complex *C. perniciosa* x *Theobroma cacao* pathosystem. Besides improved methods for inoculation of clones, there is a need to better understand how the variation in the pathogen relates to the resistance of the cocoa genotypes.

Introduction

The response of cocoa plants to infection by *Crinipellis perniciosa* (recently renamed *Moniliophthora perniciosa*) in Ecuador has been the object of different interpretations due to the lack of studies confirming relationships between findings under controlled conditions (i.e. greenhouse/laboratory inoculations) and field evaluations (Cronshaw and Evans 1978; Rocha and Wheeler 1985). In Ecuador, good results have been reported by Frias (1987) in developing the "belt spray" inoculation method, when comparing the average resistance of seedlings tested by this method and field infection of cultivars with known field resistance level (SCA6, SCA12, Catongo and EET400). Since then, the belt spray method was adopted routinely in Ecuador for early testing of average resistance of seedling populations. It was also used, at experimental level so far, to evaluate resistance of clonal materials (budded plants raised in the nursery). The belt spray method would allow comparing results between countries where this method is applied, i.e. in Brazil, Ecuador and Trinidad (Frias *et al.* 1995; Silva *et al*. 2001). The CFC/ICCO/IPGRI project provided the possibility to compare results between countries with the same set of material, i.e. the accessions of the International Clone Trial (ICT). However there are variations in the testing conditions between these countries. Results comparing field infection level with data from artificial inoculations are reported here.

Materials and methods

Two experiments were carried out jointly by the Plant Breeding Section and the Plant Pathology Laboratory of the Tropical Research Station "Pichilingue" of the Instituto Nacional de Investigaciones Agropecuarias (INIAP), located at 5.5 km south of Quevedo on the road to Guayaquil. The experimental fields of the station are between 70 and 120 m above sea level, with an annual average temperature of 24°C and 2100 mm precipitation.

Trial 1

Natural infection levels (cushion and vegetative brooms) of the accessions of the ICT were compared to the responses to artificial inoculation of grafted plants growing in the shade house and in the budwood garden. For the shade house plants, the belt spray method (Frias *et al*. 1995) was used with a suspension of 100 00 basidiospores/ml. In the budwood garden, young shoots up to 1.5-cm long were inoculated by applying pieces of agar with a suspension of 100 000 basidiospores/ml ("agar blocks") and covered with plastic bags containing humid paper towel to form a chamber with 100% relative humidity (Sreenivasan 1996). Bags were removed after 48 hours. Inoculations took place at the end of the rainy season when the danger of natural infection was less acute. Natural field infection was observed over a period of three years in the ICT (2001, 2002 and 2003) as well as in the budwood garden (2001, 2002 and 2004). Grafted plants of clone EET19 were used as controls in the inoculation tests. The ICT control clones were EET103 (susceptible) and CCN51 (moderately resistant).

Trial 2

The objectives of the second trial were to find out, firstly, whether the percentage of infection obtained with the belt spray method can be correlated with field infection, and secondly, whether symptomless seedlings after belt spray inoculation are more resistant than non-inoculated seedlings. Seedlings of 14 progenies were inoculated twice with the belt spray method. Inoculations were done with a suspension of 100 000 basidiospores/ml. In average, 80% of the seedlings showed infection after two inoculation rounds. A total of 139 non-infected seedlings were planted in the field besides seedlings of the same progenies grown in the nursery that had not undergone any infection. Natural level of infection (number of

brooms) was observed in 2002 on all inoculated and non-inoculated trees. The stem diameter of the seedlings observed in 2001 was used to correct the number of brooms for plant size.

Results and discussion

Trial 1

- **Observations on natural infection of the ICT accessions**

Table 1 shows data of natural infection in the field (budwood garden and ICT) as well as of artificial infection in the shade house and in the budwood garden. The field results gave a preliminary indication of the behaviour of the international clones in Ecuador in relation to witches' broom. The results in the budwood garden are considered as indicative only, because the number of plants per clone was low (3-12 budded plants) and the spacing of the plants was very dense, requiring intense pruning to keep the plants small. However, the 3 years' ICT field data are considered to be quite conclusive.

Average level of attack varied from 0.56 to 10.8 brooms per tree per year in the ICT and from 0.1 to 13.1 brooms in the budwood garden over the 3-year period. The coefficient of correlation between the budwood and field data was significant (r=0.49), especially so when the BE10 clone, which had an abnormal high level of attack in the budwood garden, was eliminated from the analysis (r=0.74). These results would suggest that observations on natural infection carried out on a relatively small number of trees may already give a relatively good indication of the level of field resistance of cocoa clones.

The LCTEEN46, MXC67, LCTEEN37I, EQX3360-3 and GU255V clones showed lower number of brooms per tree in the ICT than CCN51, the most resistant control clone, and also than the SCA6 clone. This is interesting because SCA6 is still found to be one of the most resistant cocoa genotypes in routine inoculation studies in Ecuador. The five most resistant clones in the ICT also showed low natural infection in the budwood garden. It is interesting to note that the SCA24 clone, not presented in the ICT, was the least infected clone in the budwood garden. Therefore, the use of this resistant "dwarf" clone in further breeding should be of interest. The most susceptible clones in the ICT were EET103, CATIE1000, VENC4-4, ICS43, UF676 and EET59. They were also among the most susceptible clones in the budwood garden.

- **Artificial inoculation of the ICT accessions**

The level of infection of budded plants in the shade house, using the belt spray method, was very variable. The results of a typical inoculation round are shown in Table 1. Inoculation of shoots in the budwood garden resulted in very low levels of infection. There was no apparent relationship between the results of the artificial inoculations in the shade house, nor in the budwood garden, with the natural level of infection in the field (Table 1).

The factors affecting the infection process may have led to variable responses of clonal materials after inoculation. The response of the open-pollinated seedlings of EET19 clearly varies between the two sets of control plantlets used in the shade house inoculations, one group showing only 33% infection while the other had 80%. In the budwood garden, many necrotic shoots developed after inoculation, which is an unusual response. The factors that may cause such variable reactions are not yet well understood. The first factor that may have played a role is the physiological condition of clonal shoots, which under our conditions were much more difficult to infect than growing points of young seedlings. Clearly, further studies are required to improve the conditions for inoculation of clonal plants.

Table 1. Response of budded accessions of the ICT to infection by *Moniliophthora perniciosa* using laboratory inoculation (belt spray), field inoculation (agar droplet) and natural infection in the budwood garden and in the ICT

Clone	Belt spray inoculation (example)		Shoot inoculation in the budwood garden*			Natural field infection **	
	Inoculated plants (no.)	Infected plants (%)	Shoots inoculated (%)	Necrotic shoots (%)	Brooms formed (%)	Budwood garden (3-12 plants per clone, 2001-02-04)	ICT (20-48 plants per clone, 2002-03-04)
SCA24	4	100	32	19	16	0.10	na
LCTEEN46	2	50	12	0	8	na	0.56
MXC67	9	44	na***	na	na	1.39	1.00
EQX3360-3	2	100	8	37	0	na	1.05
LCTEEN37-I	3	0	7	0	0	0.13	1.06
GU 255V	1	100	9	0	11	0.97	1.47
PA107	3	100	21	14	19	2.44	2.51
Playa Alta 2	10	20	52	4	2	3.00	3.17
PA120	3	33	35	9	3	2.37	3.29
BE10	2	50	7	29	0	13.08	3.30
SCA6	8	13	na	na	na	1.52	3.74
AMAZ15-15	6	17	na	na	na	2.03	3.89
IMC47	3	100	21	19	9	1.79	4.02
LAF1	6	33	29	22	0	3.50	5.16
GU175V	4	75	17	18	6	2.40	5.33
SPEC54-1	10	50	na	na	na	5.56	5.58
MAN15-2	8	13	na	na	na	2.66	6.58
VENC22-2	4	50	31	10	3	4.95	6.82
CATIE1000	3	67	39	13	3	9.67	7.90
VENC4-4	2	0	8	0	0	9.93	9.04
ICS43	8	13	na	na	na	3.53	9.95
UF676	13	23	na	na	na	4.40	10.75
EET59	3	67	25	0	28	8.08	10.80
EET19 (lab control 1)	12	33					
EET19 (lab control 2)	10	80	40	92	2		
EET103 (field control 1)							7.6
CCN51 (field control 2)							1.58

* Shoots were inoculated on trees in the budwood garden with the agar drop method
** Average number of brooms recorded per year, with one count per year.
*** na = material not available for the test

With regard to the erratic infections obtained in the budwood garden, climatic factors may have played a role. In Ecuador there are two well-defined seasons with regard to the epidemiology of the witches' broom disease (Rocha and Wheeler 1985). The rainy season, with fairly high temperatures (24-30°C) and relative humidity (80-90%), is the best for disease development. It is during the rainy season that most artificial inoculations are done. The dry season with a lower temperature range (20-28°C), cloudy sky and low relative humidity (~45%), affects adversely the inoculation results. However, during the rainy season the large variation in the intensity of sunlight might cause necrosis of young shoots within plastic bags. The EET19 clone showed the highest percentage (92%) of "necrotic shoots". Although it was not possible to recover the fungus from this tissue, the symptoms did not seem to be due to "sun burning", since the shoots had grown for a while after infection (i.e. without plastic bags) before dying (see Fig. 1). Therefore we can speculate that the necrosis could also be a consequence of some type of "hypersensitive" reaction due to high inoculum concentration. This aspect would require further studies.

Fig. 1. Necrotic or, possibly, a hypersensitive reaction of cocoa to artificial inoculation with *M. perniciosa.*

Trial 2

The results of the field observations carried out on the 139 inoculated and non-inoculated plants of 14 crosses are shown in Table 2. Firstly, the average results per cross from the belt spray inoculation and field infection was compared for all 278 trees. The linear coefficient of correlation between the belt spray results and the average number of brooms per tree for 13 of the progenies was positive (r=27), but not significant. However, when the field results were corrected for the size of the trees (square of the stem diameter), the correlation became significant (r=0.57). This can be explained by the fact that bigger canopies will in average receive more spores of the fungus and therefore show more brooms per tree than smaller plants. The corrected data are therefore expected to reflect better the inherent level of resistance of the crosses in the field. These results would suggest that the belt spray method can be efficient in identifying the average level of resistance of seedling progenies.

However, the average number of brooms per tree for the symptomless inoculated seedlings was very close (4.09) to that of the non-inoculated seedlings (3.93). These results suggest that the seedlings that do not show symptoms after the belt spray inoculation are not necessarily more resistant to the disease. In other words, these seedlings are apparently escapes rather than more resistant.

Conclusions

Field observations

Field observations seem to be the most reliable basis for selection of resistance to witches' broom. Observations on a relatively small number of plants can provide quite reliable results, as shown in Trial 1 when comparing the budwood and field data of the ICT accessions. Therefore, field resistance is apparently quite heritable. However, when plant size varies (due to different age or different genetic vigour) between genotypes, corrections of the broom number need to be made in order to obtain more reliable and heritable field data. Indeed, in Trial 2, only corrected field data showed to be significantly correlated with the belt spray inoculation results.

Artificial inoculation of seedling and clonal materials

Trial 2 has shown that the belt spray inoculations can provide a useful estimate of the average level of field resistance of seedling progenies. This is an important conclusion, which is not always easy to demonstrate. A favourable aspect has probably been that the infection data presented in Table 2 have been obtained in the same inoculation experiment. This is often not possible, because pods are generally not available at the same time, which in turn is related to differences in flowering periods and ripening dates for different cocoa genotypes. A solution to this problem that is worth trying is to synchronize the seedlings of different ages by pruning, and by using only growing points of a similar stage of development.

Table 2. Number of brooms observed in the field of belt spray selected seedlings (symptom-free inoculated seedlings) and of non-inoculated seedlings of 14 crosses

Crosses	Artificial inoculation	Natural infection in the field in 2002					Mean square of stem diameter in 2001 (cm^2)	Mean no. of brooms / square stem diameter
		Inoculated seedlings		Non-inoculated seedlings		Mean no. of brooms		
	Plants infected (%)	No. of plants	Mean no. of brooms	No. of plants	Mean no. of brooms			
CCAT46-68 x EB22-25	9.5	15	0.8	15	0.9	0.85	3.4	0.25
CCN51 x EET462	29.8	4	8.3	4	4.3	6.30	14.1	0.45
CCAT49-98 x CCAT18-58	20.8	9	0.6	9	1.3	0.95	2.1	0.45
CCN51 x EET450	19.2	14	4.0	14	2.9	3.45	7.5	0.46
EET450 x EET416	22.7	14	3.8	14	2.3	3.05	6.6	0.46
CCN51 x EET233	11.3	15	1.8	15	2.1	1.95	3.5	0.56
CCN51 x EET451	48.8	10	2.7	10	2.1	2.40	4.1	0.59
CCN51 x EET416	13.7	20	7.6	20	6.7	7.15	7.5	0.95
CCN51 x EET387	31.2	9	9.7	9	8.3	9.00	8.5	1.06
EET451 x EET450	(no data)	4	3.2	4	6.0	4.60	4.2	1.10
EET450 x EET462	51.5	7	5.0	7	6.0	5.50	4.3	1.28
CCAT46-68 x CCAT18-48	36.2	6	4.5	6	4.3	4.40	2.9	1.52
CCN51 x EB22-25	32.2	10	2.3	10	2.4	2.35	1.4	1.68
EET19 x EET48	43.5	2	3.0	2	5.6	4.30	2.3	1.87
Total		139		139				
Mean	28.4		4.09		3.93	4.01	5.09	0.79

With regard to artificial inoculation of clonal material, we have to admit that so far we have not been successful to obtain high enough levels of infection under our conditions. Besides, frequent necrosis of inoculated shoots has been observed in the shade house as well as in the budwood garden. Solutions that may be tried to overcome these problems are the following:

- For successful infection of clonal plants, the synchronized outgrowth of a large number of young shoots in the right physiological stage appears to be the first condition to be met to obtain adequate levels of infection. This may be obtained by improving the growing conditions in the shade house, so that the clonal tissue is in a more favourable condition for infection.
- A reduction in inoculum concentration may be necessary to avoid the necrotic response of the tissue when inoculations are carried out during the rainy season. This should be verified by carrying out experiments with different inoculum concentrations in the dry and rainy seasons.

- Although our experience suggests that inoculation of the first "flush" is best for seedlings while that of the third flush is best for clones, the possible effect of the type of shoots used for inoculation on the response of the tissue to infection would deserve to be studied in more detail.
- The promising results recently obtained in Trinidad and Tobago (Surujdeo-Maharaj *et al*. 2004) by inoculating clones and seedlings with the agar droplet method warrant the need for this method to be studied in more detail also in Ecuador.

Comparison of results obtained by artificial inoculation and field infection

Field attack of the fungus involves infection of vegetative parts, of cushions and of pods. Some genotypes appear to be producing more cushion brooms than vegetative brooms and vice versa. The artificial infection in the laboratory or greenhouse always involves only vegetative material, and may therefore not represent well the level of susceptibility to cushion infection. Once the method for artificial inoculation of clones is improved, it would be of interest to compare clones that have a tendency to produce more or less cushion brooms in the field.

Another aspect to be considered is the expected large variation in the pathogen population, both in time and in space. This may affect correlations between artificial inoculation and field results, if the fungal population in the field is different from that used for the artificial inoculations. Once the inoculation of cocoa clones is mastered effectively, the possible interactions between pathogen isolates and cocoa clones can be studied more easily.

Lessons learned

The final lesson learned is that a more discriminating early test for WB is required, especially to detect resistance of individual seedlings and of clonal plants. The belt spray method, as used here, can be efficient to identify the average level of resistance of seedling progenies. But even for this test the logistics need to be improved in order to obtain useful results.

The development of a more efficient early screening test to evaluate resistance of cocoa against witches' broom and moniliasis has been incorporated as a priority for the second phase of the project. Due to the importance of these studies, a student has been appointed full-time to this task.

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Gene-for-gene interactions in the *Theobroma cacao-Crinipellis perniciosa* pathosystem?

M.W. Shaw and A.E. Davies

School of Plant Sciences, The University of Reading, Whiteknights, Reading RG6 6AS, UK

Abstract

Third-country inoculations of isolates from three locations in South America onto a range of clones revealed a range of susceptibilities, and suggested that latent period might be a proxy for resistance. There was a very strong differential interaction between the resistant clone SCA6, a susceptible Amelonado clone (B9/G3) and two isolates of *Crinipellis perniciosa*. A Brazilian isolate from Rondonia State infected both clones equally; a Trinidadian isolate was able to infect only the Amelonado. Seedlings from a cross of the two clones were approximately equally susceptible to both isolates.

Introduction

If there are gene-for-gene interactions between cocoa and *Crinipellis perniciosa* (recently renamed *Moniliophthora perniciosa)*, then resistant clones selected at one location could be at risk of breakdown in other locations or if isolates of *C. perniciosa* moved from one region to another. Checking this by transferring clones between test centres can confound environmental differences with differences in the pathogen population, so it is desirable to do side-by-side testing of clones with isolates from a variety of geographic provenances. For obvious reasons, this has to be done outside the cocoa-producing areas, which requires an artificial greenhouse inoculation system. We therefore had three aims: (i) to develop a greenhouse inoculation method to test defined, genetically pure isolates on cloned cocoa; (ii) to use this to test clone-isolate interactions in one location; and (iii) to confirm by an independent method the results of bulk clone screening (Frias 1987) in producing regions of South America. The key results will appear in detail shortly (Shaw and Vandenbon 2006).

Materials and methods

Isolates were multiplied on artificial media by the method of Griffith and Hedger (1993) and basidiospores collected in 16% glycerol solution and frozen until needed. About 35 isolates were used in initial work; isolates reliably producing crops of well-germinating spores were used in preliminary work. The bulk of the study used three isolates: Castanhal-I (Cast-I; via Silwood Park/B.E. Wheeler, isolated from Rondonia, Brazil, in an area of SCA6 resistance breakdown), Gran Couva-A5 (GC-A5; via Silwood Park/B.E. Wheeler, isolated from Trinidad) and APC3 (via Aberystwyth/G.C. Griffiths, isolated from Bahia, Brazil).

Inoculation by spray, weak agar drop, water drop, agar block, and direct application of basidiocarps were compared, in an enclosed chamber continuously humidified with an atomizer. The eventual method chosen was to apply four drops, each of 4 µl, of a suspension of spores in water onto developing buds, when conditions outside the greenhouse favoured dew formation in the moist chamber, i.e. there was a clear sky. Spores were applied at a rate of 20 000 per bud. This method gave a high proportion of successful inoculations; in contrast to other methods, the dose applied was easy to quantify, and the success of inoculation was easy to score.

Results

There was a highly significant inverse relationship between the percentage success of an inoculation of a particular host with a particular isolate on a specific occasion, and the latent period until broom formation, accounting for about half the variance in latent period across the series of experiments. There was an average period of 6 weeks between inoculation and symptom appearance in inoculations with success rates around 50% of buds inoculated, but about 10 weeks for the less compatible combinations with success rates around 10%. It is unclear whether this is due to correlation between components of resistance, or because fewer successful inoculations also implies fewer initial infection points on one broom and some sort of cooperation between multiple infecting spores.

Infection success, averaged over isolates, varied between clones (Fig. 1). The results are broadly in line with those obtained by other methods, and field observations.

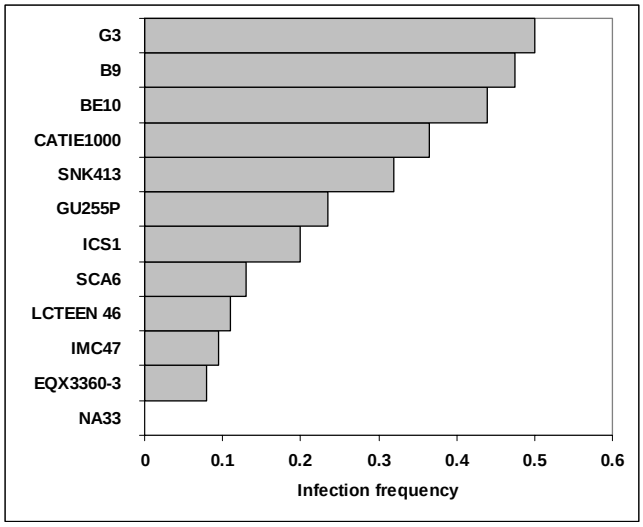

Fig. 1. Frequency of infected buds in various clones. Data are corrected for differences between inoculations by generalized linear modelling using clones appearing in multiple inoculations. Sample sizes differ widely so no single SEM (standard error of the mean) applies.

Two types of interaction were noted. First, there was a weak correlation between the latent periods (corrected for differences between inoculations) of brooms produced by isolates Gran Couva A5 (Trinidad) and Castanhal-I (Rondonia) on a range of clones susceptible to both. Second, there was a very strong host-pathogen interaction between these two isolates and the host pair Amelonado B9 (or G3) and SCA6. Castanhal-I was clearly pathogenic on both, whereas in over 100 inoculations Gran Couva A5 never infected SCA6 despite being slightly more pathogenic on the susceptible host, with an average success rate of 52%. This difference was present with either a 24-h or a 70-h infection period in the high humidity environment. Progeny testing of individual seedlings of a G3 x SCA6 cross with both isolates (at least two inoculations of each seedling with each isolate) suggested that all progeny were more or less equally susceptible to both isolates, so this effect is not attributable to a classic dominant R-gene. It appears to be different from the gene identified by Queiroz *et al.* (2003) because this was dominant. However, Brown *et al.* (2005) identified a minor QTL (quantitative trait locus) with recessive resistance; if the inocula relevant to their resistance data contained a proportion of Gran-Couva A5 type isolates, this QTL could be the same as the factor in this cross.

Conclusions

Third-country laboratory-based tests for resistance to *C. perniciosa* are possible and give results comparable to field tests, but are very expensive in both labour and facilities. They could not be of use in practical breeding, and should be considered only when there is no other way to achieve a scientific goal. Latent period to symptom or broom production is well correlated with infection efficiency, which may be useful in designing short-cut resistance tests (Surujdeo-Maharaj *et al*. 2003). Specific host genotype x pathogen genotype interactions exist, so there is a serious risk of varietal breakdown if single resistant clones with uncharacterized resistance are grown over large areas, especially in monoculture. Genes liable to breakdown could include QTLs of large effect.

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MIRID RESISTANCE STUDIES

Activities carried out to evaluate cocoa resistance to mirids (*Sahlbergella singularis*) at IRAD, Cameroon

R. Babin[1], J.M. Mpé[2], L. Dibog[2], J. Amang à Mbang[2], S. Nyassé[2] and A.B. Eskes[3]

[1] CIRAD, Direction Régionale, BP 2572, Yaoundé, Cameroon

[2] IRAD, BP 2076, Yaoundé, Cameroon

[3] IPGRI/CIRAD, c/o INIBAP, Parc Scientifique Agropolis II, 34397 Montpellier cedex 5, France

Abstract

Field and laboratory tests were conducted for different components of the resistance of cocoa to mirids (*Sahlbergella singularis*) at the Nkoemvone and Nkolbisson research stations of IRAD as part of the CFC/ICCO/IPGRI project activities in Cameroon. Almost 40 local or international genotypes were tested for one or for several components of resistance. Laboratory micro-tests were used to assess attractiveness. Antixenosis was measured by enclosing mirid nymphs in sleeve cages on young flushes. Attractiveness and antixenosis were estimated in the same set-up by counting the feeding points after 24 hours. Ability to recover from mirid damage (tolerance) was assessed by observing degradation of twigs with time. Finally, antibiosis tests were conducted with the aphid *Toxoptera aurantii*, with the aim of evaluating whether this aphid could be used as an indicator for cocoa antibiosis towards mirids.

Statistical analysis generally revealed a strong effect of genotypes on the different components of the resistance. However, there was no significant difference between the genetic groups studied (Upper Amazon and Lower Amazon Forasteros, Trinitarios and hybrids), which appears to be in contrast with field observations on mirid damage in Côte d'Ivoire. We also failed to identify genotypes that combine favourable responses to several resistance components. As an important result, the activities have made it possible to improve and standardize the evaluation methods. Mechanisms involved in attractiveness and antixenosis appeared to be similar, therefore one test should be enough to evaluate these components. We propose to standardize the methods for antixenosis (under no-choice conditions) and tolerance. We suggest that a new assessment method be developed for the effect of genotype on antibiosis (female mirid fecundity and nymph survival), using cocoa pods on the tree as the feeding and egg-laying environment. Finally, we propose to include in our future activities studies on resistance of cocoa to fungal pathogens that may be associated with mirid damage, with the aim of achieving a better understanding of tolerance and dieback.

Introduction

In the past, several studies have been devoted to measuring the tolerance of cocoa trees to mirids (*Sahlbergella singularis* and *Distantiella theobromae*) by observing cumulated and recent damage in field trials in Cameroon and Côte d'Ivoire (Decazy and Lotodé 1975; Decazy and Coulibaly 1982; Sounigo *et al.* 1994). This method permits ranking of genotypes according to their global reaction to mirid attacks. But it involves natural infestation in experimental fields, which is generally undesirable and does not permit early screening of genotypes. Mechanisms involved in plant resistance to an insect are complex, and this is particularly true for cocoa mirids. Plant attractiveness affects the level of infestation, antixenosis prevents mirid feeding while antibiosis disturbs mirid development, and finally cocoa tolerance is linked to the ability of a tree to contain damage and recover from it.

The Laboratory of Entomology of the Institut de Recherche Agricole pour le Développement (IRAD), located in Nkolbisson, near Yaoundé, develops research activities with the aim of gaining a better understanding of resistance mechanisms and of identifying cocoa genotypes with resistance and/or tolerance to mirids (*S. singularis*) in Cameroon. The goal of this paper is to present the different methods used, results obtained and problems encountered in the studies carried out by IRAD and CIRAD scientists with partial support from the CFC/ICCO/IPGRI project.

Materials and methods

Plant material

The genotypes tested are listed in Table 1.

Table 1. List of genotypes of different genetic groups tested for mirid resistance components (At = attractiveness, Ax = antixenosis, To = tolerance and Ab = antibiosis)

Upper Amazon Forastero	Resistance components evaluated	Lower Amazon Forastero	Resistance components evaluated	Trinitario	Resistance components evaluated	Hybrids*	Resistance components evaluated
PA7	Ax To	SIC5	At Ax To	ICS1	Ax To Ab	SNK614	At Ax To
PA107	At	BE10	At Ax To	ICS43	Ax To	SNK619	At Ax To Ab
NA33	Ax To	Catongo	At Ax To Ab	UF676	Ax To	SNK620	Ax To
SCA6	Ax To	IFC5	Ax To	SNK16	Ax To	SNK622	Ax To Ab
T60/887	At Ax To Ab	IFC1362	Ax To	SNK30	Ax To	SNK625	Ax To
T79/501	Ax To	IFC1373	Ax To	SNK64	Ax To	SNK633	Ax To
IMC60	At Ax To	IFC1374	Ax To	SNK413	At Ax To Ab		
UPA134	At Ax To Ab	IFC1375	Ax To	SNK416	Ax To		
UPA143	Ax To	IFC1376	Ax To	SNK600	Ax To Ab		
GU255V	Ax To			SNK608	Ax To		
SPEC54-1	Ab			Playa Alta 2	At Ax To		
MO20	Ab						

* Upper Amazon Forastero x Trinitario

Attractiveness

The method used was adapted from the laboratory micro-test of Nguyen-Ban (1998). Three green twig segments of 5 cm in length and with roughly similar diameter were arranged in a triangle, stapled together on a filter paper and placed in a 9-cm diameter Petri dish. Each genotype was tested three times in a circular design test. Fifteen twig segments were used per genotype in each test, so 45 observations were carried out per genotype. The use of control genotypes was therefore not necessary. A starved mirid nymph (fourth or fifth stage) of *S. singularis* was introduced into each Petri dish, and 24 hours later mirid feeding points were counted on the twig segments.

Antixenosis and tolerance

Antixenosis is a defence mechanism of the plant that leads to a non-preference (when infestation is natural) or a non-acceptance (when infestation is artificial) of the plant as a feeding source by the insect (Painter 1958). By these tests we tried to measure the mirid acceptance of different cocoa genotypes as a feeding source. Contrary to attractiveness, antixenosis was in this case assessed by non-choice tests, on adult cocoa trees. Activities were conducted with 28 genotypes from the IRAD clone collection at the Nkoemvone station

between March 2001 and March 2002, and with 10 genotypes from the budwood garden containing introduced and local clones at the IRAD station at Nkolbisson in 2003.

At the Nkoemvone station, mirid nymphs of the first to fourth stages were collected early in the morning and confined on a young flush in a nylon mesh sleeve cage. Five trees for each clone were tested with six observations (flushes) per tree. The number of feeding points was counted every 24 hours for five days after infestation. Mirids were kept on the flushes until they died. The damage caused by attacks was then assessed with the help of a 5-point scale, with 0 = dead and 4 = completely healthy flush. A "flush degradation rate" was calculated by the difference between the scores at the start of infestation and the score at the moment the nymph died (or the adult appeared), divided by the number of days of observation.

The method used at the Nkolbisson station has been presented in detail by Babin *et al.* (2004). Mirid nymphs (stage 5) came from laboratory rearing. Eight to 14 observations per genotype were carried out. A starved mirid nymph was confined on a flush in a nylon mesh sleeve cage and allowed to feed on the plant for 24 hours. Then the nymph was removed and the number of feeding points counted. The ability to contain damage and to recover from damage was assessed by observing the reaction of the flush to damage and the ability of the twig to sprout (at the base or at the end of the flush). The reaction of the twig was examined twice a week for three weeks, by using a scale ranging from 0 (healthy flushes) to 4 (dead flushes). For each flush, the mean damage value was calculated for all the scores given at the different observation dates. This way, the mean damage score takes into account the speed as well as the final degree of damage. One month after infestation and at the end of the dry season, i.e. 8 to 9 months after infestation, the ability of the twig to recover from damage was estimated by observing sprouting. Each twig was assigned to one of the following classes: 1) the branch was sprouting normally; 2) the flush had dried but the branch was sprouting at its base; 3) the branch was completely dry (dieback). A rate of sprouting was then calculated for each genotype. Flush age and size were assessed before the test.

Antibiosis towards mirids

The method consisted of following the development of a mirid nymph confined on a twig in a nylon mesh sleeve cage. Because of the great damage caused by individual mirids on twigs, it was difficult to assess antibiosis of cocoa towards mirids by feeding the mirids on cocoa twigs. This method was therefore abandoned.

Antibiosis towards aphids

The aim was to evaluate whether aphids can be used as indicator insects to assess the reaction to mirids. These activities were conducted at Nkolbisson, on young grafted plants in the nursery and in the budwood garden (5-year-old trees). Adult aphids (*Toxoptera aurantii*) were collected from nearby cocoa trees and confined on young cocoa leaves in clip-cages. The next morning, the larvae produced were counted and only one of them was kept in the clip-cage, while adults and other larvae were removed. Nymph development was monitored daily until the adult stage. Fecundity of the adult was then examined daily until its death, newly born larvae being counted and removed each day. Life tables were established by observing 4 to 11 aphids per genotype of the host plant. A life table is a listing of the number of individuals in a population surviving at specific ages in the life cycle (Elkinton 1994). It allowed the calculation of an intrinsic rate of natural increase (r) for each genotype. Intrinsic rates of natural increase were calculated with several fecundity and survival parameters and therefore could not be statistically compared. However, they are regarded as sound indicators of antibiosis.

Results and discussion

Effect of genotype on resistance reactions

Statistical analyses did not reveal any effect of genotype group (Trinitario, Upper Amazon, Lower Amazon and "hybrid" clones) on the different components of resistance. However, the analyses always showed significant differences between individual genotypes for the different resistance components (Tables 2 to 5).

Attractiveness and antixenosis

Despite the fact that the methods were different, ranking showed similar results for attractiveness and antixenosis tests conducted at Nkolbisson (Table 2). Indeed for these two components IMC60, Catongo and SIC5 were among the least susceptible genotypes while BE10 was among the most susceptible. The mechanisms involved in these two components may be similar. The laboratory micro-tests showed that every tested genotype revealed at least some mirid feeding points, suggesting that mirids probably made their preference only after tasting the twigs. These observations suggest that laboratory micro-tests do not only measure attractiveness but also antixenosis under free-choice conditions (feeding non-preference). Insofar as several mechanisms of resistance are involved in micro-tests, their interpretation seems to be more difficult and their application in breeding programmes not easy. The method used for antixenosis assessment has the advantage that it also allows the assessment of tree tolerance. However, this field test requires availability of large trees, resisting attack of mirids, and care needs to be taken to avoid possible spread of mirids to neighbouring trees.

Table 2. Ranking of genotypes for attractiveness to mirids estimated in the laboratory and for antixenosis to mirids tested in the field (Nkolbisson). For antixenosis, the number of feeding points after 24 hours has been corrected by the estimated flush-leaf surface area used as a covariable.

Attractiveness (Nkolbisson)			
Genotype	**Feeding points mean number**		**N***
IMC60	2.44	a ***	45
Catongo	2.56	ab	45
Playa Alta 2	2.69	ab	45
SIC5	2.87	ab	45
SNK614	2.98	ab	45
PA107	3.09	ab	42
SNK619	3.20	ab	45
UPA134	3.55	abc	42
T60/887	3.60	abc	45
SNK413	4.45	bc	42
BE10	4.96	c	45
Anova	F=3.15	P<0.001	

Antixenosis (Nkolbisson)			
Genotype	**Feeding points mean number ****		**N**
SIC5	9.01	a	11
Catongo	9.04	a	14
UF676	9.93	ab	13
IMC60	10.63	ab	11
ICS1	11.43	abc	14
T79/501	11.77	abcd	11
Playa Alta 2	12.23	bcd	10
BE10	12.92	bcd	8
GU255V	14.09	cd	11
IFC5	14.48	d	10
Ancova **	F=2.94	P<0.001	

* N = number of observations

** Analysis of covariance with the estimated flush-leaf surface area used as a covariable

*** Genotypes followed by the same letter do not show significant differences according to the pairwise Student T-test at a 5% significance level

Antixenosis and tolerance

Studies of antixenosis and tolerance at Nkoemvone (Table 3) gave interesting ranking results but they were quite different from those of the Nkolbisson studies (Table 2). For instance, ICS1 was a better genotype than IMC60 for antixenosis according to Nkoemvone tests but these accessions were not different at Nkolbisson. Meanwhile ICS1 was assessed as more tolerant than T79/501 at Nkoemvone but was not different at Nkolbisson. These results are consistent with the suggestion that ICS1 was represented by different genotypes at Nkolbisson and Nkoemvone (indicated by molecular studies). The differences between the methodologies applied could also be an explanation. Indeed, the method used at Nkoemvone for antixenosis and tolerance was modified for different reasons. After mirid feeding, a well-marked black lesion develops. The number and the size of the lesions may vary according to the origin and the development stage of the mirid. Consequently, it was necessary to infest flushes with a homogeneous population of mirids of approximately the same age. When feeding points are numerous, black lesions overlap and it becomes difficult to count them. In addition, the presence of too many lesions prevents mirids from feeding. For these reasons, mirids should not be confined on flushes for more than 24 hours.

Table 3. Ranking of genotypes for antixenosis and tolerance evaluated in the field at Nkoemvone. Genotypes are ranked from the least to the most susceptible for antixenosis according to the number of feeding points on a flush after 24 hours and for the rate of degradation of flushes, corrected for the number of feeding points used as a covariable.

Antixenosis (Nkoemvone)			
Genotype	**Feeding points mean number**		**n**
IFC1373	23.5	a **	22
SNK413	24.4	a	14
SNK625	28.6	ab	28
SNK64	30.7	abc	18
T60/887	33.7	abcd	22
SNK622	34.6	abcd	23
SNK416	35.2	abcd	26
SNK608	35.4	abcd	23
SNK30	35.5	abcd	11
ICS1	36.3	abcd	23
UPA143	36.6	abcd	21
SNK619	37.1	abcd	15
UPA134	38.8	abcd	20
SNK633	39.7	abcd	23
IFC1362	39.8	abcd	21
SNK16	41.4	abcde	12
NA33	42.2	abcde	22
SNK620	43.6	abcde	23
SCA6	44.3	abcde	19
SNK600	52.0	bcdef	15
PA7	53.3	cdef	27
IFC1374	54.9	def	16
IFC1376	61.6	efg	22
SNK614	68.3	fg	20
IMC60	72.9	gh	26
IFC1375	87.3	hi	22
ICS43	88.1	hi	25
T79/501	96.2	i	27
Anova	F=12.3		P<0.01

Tolerance (Nkoemvone)			
Genotype	**Degradation rate ***		**n**
SNK633	a		23
SNK416	ab		26
SNK619	abc		15
IMC60	abc		26
IFC1362	abc		21
ICS1	abc		23
SNK413	abcd		14
SNK625	abcd		28
IFC1376	abcde		22
PA7	abcde		26
SNK620	abcde		23
UPA134	abcdef		23
SNK622	bcdef		23
T60/887	bcdef		22
IFC1373	cdefg		22
SNK30	cdefg		11
SNK608	defg		20
SNK614	defg		20
SNK16	defg		12
SNK64	defg		18
T79/501	defgh		27
ICS43	efgh		25
IFC1374	efgh		16
NA33	fgh		22
UPA143	gh		21
SCA6	hi		19
IFC1375	i		22
SNK600	i		15
Ancova*	F=6.40		P<0.01

* Analysis of covariance with mean number of feeding points used as a covariable

** Genotypes followed by the same letter do not show significant differences according to the Duncan test at 5% significance level

Aphid antibiosis

The results of the experiment are shown in Table 4. The ranking of intrinsic rates of natural increase in the aphid populations showed that PA7 had the greatest ability to prevent aphid development for the test carried out in the budwood garden (5-year-old trees). On grafted young plants in the nursery UPA134 showed the strongest antibiosis. The orders of aphid antibiosis ranking (Table 4) and mirid tolerance ranking at Nkoemvone (Table 3) seem to be similar, while antixenosis ranking at Nkoemvone (Table 3) seems to be negatively correlated with aphid antibiosis (Table 4).

The mechanisms involved in these components of the resistance may be different. Antibiosis observation showed that one of the main factors influencing aphid survival and reproduction may be the duration of young terminal leaf development. Indeed, lignification of leaves seems to reduce or prevent aphid development. Degree of twig lignification at the time of attack may therefore also be an important factor in cocoa resistance/tolerance to mirids. However, this factor is not easy to measure for mirids; the damage caused to the twig is so great that it leads to the starvation and death of the insect in a few days. Assessment of female fecundity and nymph survival rate may be more easily assessed on cocoa pods, for which damage is more moderate. A method of assessment based on pod infestation is currently being tested at Nkolbisson station.

Table 4. Ranking of genotypes for antibiosis evaluated at Nkolbisson with regard to aphids. Genotypes are ranked from the most to the least resistant, according to the intrinsic rate of natural increase (r) of the aphid population.

Antibiosis to aphids (Nkolbisson)			
Genotype	**Test location**	**r**	**n**
PA7	Budwood garden	0.325	10
SPEC54-1	Budwood garden	0.326	9
NA33	Budwood garden	0.329	11
UPA134	Nursery	0.334	4
MO20	Budwood garden	0.338	44
SNK619	Nursery	0.341	7
ICS1	Nursery	0.344	9
SNK413	Nursery	0.347	4
SNK622	Nursery	0.362	6
T60/887	Nursery	0.367	4
Catongo	Nursery	0.367	10
SNK600	Nursery	0.368	8

r = intrinsic rate of natural increase
n = number of observations

Tolerance

Results of the tolerance assessment obtained at Nkolbisson station (Table 5) showed that the ability to contain damage (mean damage) and the ability to recover from it (sprouting rate) were probably two distinctly different components of tolerance. IMC60 and UF676 were the most tolerant genotypes based on their ability to contain the evolution of damage. But IMC60 showed a relatively low percentage of sprouting of damaged branches after the dry season. On the other hand, ICS1 was the least tolerant genotype based on mean damage but the damaged branches showed good sprouting capability after the dry season.

Table 5. Ranking of genotypes for mirid tolerance evaluated at Nkolbisson. Genotypes are ranked according to their ability to contain damage (mean damage scores corrected for the mean number of feeding points) and for the ability of the twigs to recover from damage (% of twigs sprouting one month after infestation and at the end of the dry season, i.e. 8 to 9 months after infestation).

Tolerance (Nkolbisson)*								
Genotype	**Mean damage****		**Genotype**	**Sprouting % (after 1 month)**		**Genotype**	**Sprouting % (end dry season)**	
IMC60	2.21	a***	Playa Alta 2	100	a	ICS1	93	a
UF676	2.28	a	ICS1	93	b	UF676	92	a
T79/501	2.51	ab	Catongo	93	b	Playa Alta 2	90	ab
GU255V	2.60	abc	T79/501	91	bc	IFC5	90	ab
Catongo	2.71	bc	SIC5	90	bc	GU255V	82	bc
SIC5	2.78	bc	IFC5	90	bc	SIC5	80	c
Playa Alta 2	2.80	bc	UF676	85	c	T79/501	64	d
IFC5	2.90	bc	GU255V	82	c	IMC60	64	d
BE10	2.96	bc	IMC60	73	c	Catongo	64	d
ICS1	2.97	c	BE10	37	d	BE10	25	e
Ancova **	F=3.57	P<0.001	Chi^2=207.33	P<0.0001		Chi^2=210.20	P<0.0001	

* For number of observations per clone see Table 2 (Antixenosis Nkolbisson)
** Covariance analysis with the mean number of feeding points as the covariable
*** Genotypes followed by the same letter do not show statistically significant differences

General discussion

The results show that the mechanisms involved in cocoa resistance to mirids are numerous and complex. The assessment methods did not allow us to rank the genotype groups (Upper Amazon, Lower Amazon, Trinitario and hybrids) for the different components of cocoa resistance to mirids. This is somewhat unexpected, as field observations in Côte d'Ivoire suggest that Trinitario as a group is more susceptible than Upper Amazon as a group (Sounigo *et al.* 1994; N'Guessan *et al.*, pp. 170-176, this volume).

Significant variations for resistance components were observed between individual genotypes. It was, however, not possible to identify a single clone accumulating favourable responses for all resistance components. For example, IFC1373 and SNK413 may be considered as promising genotypes for antixenosis tests conducted at Nkoemvone, whereas SIC5 and Catongo displayed the best results for the antixenosis tests conducted at Nkolbisson. According to the ability to recover from damage (% sprouting), Playa Alta 2, ICS1 and UF676 should be considered as the most promising genotypes.

Comparison of our current results with field observations on mirid damage carried out in Côte d'Ivoire (Sounigo *et al.* 1994) is not easy. However, comparison is possible for some genotypes. T79/501 is one of the less damaged Upper Amazon Forastero genotypes in Côte d'Ivoire. In our studies, it showed the highest number of feeding points (low antixenosis) in the tests conducted at Nkoemvone. On the other hand, T79/501 is ranked among the medium genotypes for antixenosis assessed with the micro-test method (Nkolbisson) and among the most tolerant genotypes for ability to contain damage. Thus, T79/501 could be attractive to mirid feeding but tolerant to damage. The Lower Amazon Forastero clone IFC5 is among the more damaged genotypes in the Côte d'Ivoire field study. This agrees with our results, as it ranked among the most susceptible genotypes for antixenosis and among the least tolerant genotypes at Nkolbisson. The Catongo clone is especially susceptible to dieback in Côte d'Ivoire. Tested at Nkolbisson it showed good results for antixenosis but was relatively susceptible to mirid attack, showing low ability for sprouting of damaged twigs, especially after the dry season. This seems in agreement with the Côte d'Ivoire field observations.

As a result of this work, the test methods have been improved and standardized. In future work, we propose not to use the laboratory test for attractiveness. Instead, we propose to

work mainly with the methods for antixenosis (under no-choice conditions) and tolerance (ability to contain damage and to recover from it by sprouting) as applied in the current research in the budwood garden at Nkolbisson. We also suggest developing a new assessment method for evaluating the effect of genotypes on female fecundity and nymph survival, using cocoa pods on the tree as the feeding and egg-laying environment. Finally, some studies showed that associated fungi could be related to severe forms of dieback of branches or of entire trees. These associations and the susceptibility of genotypes to these fungi are being studied in more detail at Nkolbisson, with the aim of achieving a better understanding of the phenomenon of dieback.

Antixenosis, antibiosis and tolerance of trees to mirids and associated fungi may be the most suitable traits to take into account when studying resistance of cocoa trees to mirids. It is yet to be identified which of these traits are the most important to explain accumulated damage caused by mirids in the field, and hence the most adequate traits to be measured as routine selection criteria in breeding programmes.

Acknowledgements

The authors thank the technical assistants of the entomology laboratory of IRAD stations at Nkolbisson and at Nkoemvone who performed the different tests and observations.

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Mirid resistance studies in Côte d'Ivoire: field observations on recent and cumulative damage

K.F. N'Guessan[1], A.B. Eskes[2] and P. Lachenaud[3]

[1] CNRA, BP 808, Divo, Côte d'Ivoire
[2] IPGRI/CIRAD, c/o INIBAP, Parc Scientifique Agropolis II, 34397 Montpellier cedex 5, France
[3] CIRAD-CP, TA80/02, Avenue Agropolis, 34398 Montpellier cedex 5, France

Abstract

A study was conducted in Côte d'Ivoire with the objectives of firstly, classifying the major genetic groups of cocoa based on the level of resistance or susceptibility to cocoa mirids, and secondly, identifying promising genotypes within each genetic group. All the genotypes were evaluated on the basis of recent and cumulative mirid damage in a field collection. A score of 0 (no damage) to 4 (severe damage) was given to each genotype. The results showed that there were significant differences between the major genetic groups of cocoa with regard to mirid damage. The Upper Amazon, wild Guianan and hybrid genotypes were the least damaged by the mirids. The scores of these groups of cocoa varied between 0.1 and 0.3 for the recent damage; and between 0.4 and 0.8 for the cumulative damage. The Catongo group was by far the most susceptible group. This group had scores of 2.3 for recent damage and 2.0 for cumulative damage. The Amelonado, near Criollo and Trinitario groups were also found to be susceptible but to a lesser extent. Promising genotypes were identified within each group.

Introduction

The cocoa mirids, *Sahlbergella singularis* and *Distantiella theobromae* are two major insect pests of cocoa in Côte d'Ivoire as well as in other cocoa-producing countries in West Africa. These insects may cause damage to all parts of plant except to the leaves and the roots. The most serious damage is inflicted on the trunk, the young shoots and the developing fruits (cherelles). This damage may result in 30 to 40% yield loss and partial or total degradation of plantations (Lavabre 1977a).

In Côte d'Ivoire, cocoa mirids are controlled by combining agronomic practices and insecticide applications (Lavabre 1960; Marchart 1971; Nguyen-Ban 1971; Decazy 1979; Decazy and Essono 1979; Coulibaly *et al*. 1998). However, many problems are associated with chemical use. First of all, farmers have been reluctant to adopt chemical control because of the high cost of chemicals and application equipment. Other problems are related to environmental contamination, effects on non-target organisms and potential residues in cocoa beans.

In order to develop alternative control methods, research has also been oriented towards the search for resistant cocoa genotypes. A number of studies have been carried out with regard to the development of screening methods and the evaluation of plant material for resistance to mirids (Bruneau de Miré and Lotodé 1974; Decazy and Lotodé 1975; Decazy and Coulibaly 1982; Nguyen-Ban 1994). However, progress so far is quite limited. The current breeding programme in Côte d'Ivoire, based on reciprocal recurrent selection, has been taking into account cocoa resistance to mirids and black pod disease (*Phytophthora* pod rot or Ppr). The choice of the genotypes making up the initial population was based on the available germplasm held in the collection of the Centre National de Recherches Agronomiques (CNRA) in Divo. Main traits considered were general combining ability for yield and resistance to diseases and

pests. The present study was the initial step in the characterization of the different genotypes planted in the germplasm collection in Divo for field resistance to mirids.

Materials and methods

The study was conducted at the CNRA research station in Divo, Côte d'Ivoire. The materials tested were genotypes planted in a plot in the 1980s (a few genotypes were added in the 1990s). These materials are from various origins. Some were introduced from Latin American countries and belong to the major genetic groups of cocoa such as Upper Amazon (mainly Pound collections), Amelonado, "Criollo" (Trinitario types near to pure Criollo), Trinitario and Catongo (near homozygous Amelonado selection from Brazil). Other genotypes belong to specific groups: (i) materials collected in the wild in French Guiana, identified as "Guianan" (Lachenaud and Sallée 1993); (ii) material from Venezuela; (iii) doubled haploids of Upper Amazon origin; and (iv) local hybrid clones, mainly selected in crosses between Upper Amazon and Amelonado clones. Four related *Theobroma* species were also included in the test (*T. grandiflorum*, *T. bicolor*, *T. microcarpum* and *T. speciosum*).

About 500 genotypes (clones) are represented in the plot. Each genotype is represented by five trees planted in a row. Before starting the observations, the plot was left without insecticide treatment for a period of one year (2000) so that it was severely attacked by the mirids. The new damage caused by the mirids is identified here as "recent damage". The recent damage was characterized by dieback of leaves and twigs in the canopy of the trees. On the other hand, observations were made on mirid damage that has accumulated over several years on the trunk and branches; such damage is identified hereafter as "cumulative damage". Cumulative damage is made up of cankers of different sizes and intensity visible on the trunks and branches. A score was given to each tree on the basis of the intensity (number and size) of these cankers on the trunks and the main branches.

Rating of cumulative damage has been used by several researchers to search for resistant cocoa genotypes (Decazy and Coulibaly 1982; Sounigo *et al*. 1994). The rating is performed on a scale of 0 to 4 where 0 corresponds to no damage, 1 corresponds to 25% of the surface of trunk and branches cankered, 2 corresponds to 50% of the surface of trunk and branches cankered, 3 corresponds to 75% of the surface of trunk and branches cankered, and 4 corresponds to almost all the trunk and branches cankered. The rating of the recent damage was performed in a similar manner but the score was given according to the level of dieback of the leaves and twigs. Thus, 0 corresponds to no dieback, 1 corresponds to 25% of the leaves and twigs showing dieback, 2 corresponds to 50% of the leaves and twigs showing dieback, 3 corresponds to 75% of the leaves and twigs showing dieback and 4 corresponds to almost all the leaves and twigs showing dieback.

Four technicians carried out the ratings on all individual trees of each genotype. Mean scores of each genotype were used to analyze variation between genetic groups. For the analyses of variation within each group, the mean score of each technician was considered as a replicate in the statistical analysis. The genotypes having at least one off-type in the row of 5 trees were excluded from the analysis.

The data were submitted to an analysis of variance using the GLM procedure of SAS (SAS Institute 1996). Mean separation was performed by the Waller-Duncan K-ratio T-test. A Pearson correlation coefficient was estimated between the cumulative and recent damage.

Results and discussion

Significant differences were found between the major cocoa groups for recent mirid damage (DF=9; F=77.9; P=0.0001) and for cumulative damage (DF=9; F=52.2; P=0.0001) (Table 1). The Upper Amazon, Guianan and hybrid genotypes were the least damaged by the mirids

(Tables 2 and 3). The average scores of these genetic groups varied between 0.1 and 0.3 for the recent damage; and between 0.4 and 0.8 for the cumulative damage; indicating that very little of their canopies showed dieback of branches and less than 25% of the surfaces of the trunks and branches were covered with cankers. On the other hand, Catongo was by far the most susceptible group. This group had scores of 2.3 for recent damage and 2.0 for cumulative damage. This indicated that the Catongo group had more than 50% of the canopy showing dieback and more than 50% of the surfaces of the trunks and branches were covered with cankers. The Trinitario, the Amelonado and the "Criollo" were also found to be susceptible but to a lesser extent.

Table 1. Result of the analysis of variance of scores of recent and cumulative mirid damage for genotypes within major genetic groups in the cocoa collection in Divo

Sources of variation	DF	Recent damage		Cumulative damage	
		F	P	F	P
Variance between genetic groups of cocoa	9	77.9	0.0001	52.2	0.0001
Variance within groups					
Catongo	15	16.8	0.0001	02.3	0.015
Trinitario	69	06.6	0.0001	03.8	0.0001
Amelonado	75	12.4	0.0001	02.6	0.0001
Upper Amazon doubled haploids	46	7.9	0.0001	01.3	0.110
"Criollo"	18	10.6	0.0001	02.5	0.0054
Venezuelan	7	3.2	0.018	04.3	0.0042
Guianan	43	10.9	0.0001	03.9	0.0001
Hybrids	49	4.1	0.0001	03.2	0.0001
Upper Amazon	103	2.5	0.0001	03.1	0.0001
Related *Theobroma* species	5	-	-	-	-

Table 2. Scores obtained for recent mirid damage for selected cocoa groups

Genetic groups of cocoa	Score for recent damage	Mean separation
Catongo	2.32	a
Trinitario	1.15	b
Amelonado	1.08	b
Upper Amazon doubled haploids	0.78	c
"Criollo"	0.75	c
Venezuelan	0.50	d
Guianan	0.32	de
Hybrids	0.30	de
Upper Amazon	0.19	ef
Related *Theobroma* species	0.00	f

Means within the same column followed by different letters are significantly different at P<0.05 according to the Waller Duncan K-ratio T-test (SAS Institute 1996)

Table 3. Scores obtained for cumulative mirid damage for selected cocoa groups

Genetic groups of cocoa	Score for cankers	Mean separation
Catongo	2.03	a
"Criollo"	1.59	b
Trinitario	1.34	c
Amelonado	0.98	d
Upper Amazons doubled haploids	0.93	d
Venezuelan	0.84	de
Hybrids	0.83	de
Upper Amazon	0.69	e
Guianan	0.39	f
Related *Theobroma* species	0.00	g

Means within the same column followed by different letters are significantly different at P<0.05 according to the Waller Duncan K-ratio T-test (SAS Institute 1996)

These results suggest that the Upper Amazon, the Guianan and the hybrid groups contain several promising genotypes for mirid resistance. Our results corroborate those of Sounigo *et al.* (1994) who showed, from a study of the behaviour of a group of parents and their progenies vis-à-vis cocoa mirids, that Upper Amazons and their progenies were more promising for mirid resistance than the other tested groups and their progenies. In our study the good performance of the cloned hybrids could be explained by the fact that the initial breeding programme in Côte d'Ivoire was founded on crosses made between introduced Upper Amazon and local Amelonado genotypes, and that these cloned hybrids were already selected for their agronomic behaviour.

A significant positive correlation (r=0.86; P=0.0001) was revealed between the average recent and cumulative damage for the genetic groups, indicating that groups showing heavy cumulative damage were also showing heavy recent damage. The same result was observed for the correlation between all individual genotypes evaluated (r=0.48; P=0.0001). Moreover, correlation analysis between the recent and cumulative damage for genotypes within each group also gave varying levels of positive and generally significant correlations (Table 4). These results indicate that genotypes which showed heavy cumulative damage are the same as those showing heavy recent damage.

Table 4. Correlation between cumulative and recent mirid damage for genotypes within different genetic groups of cocoa

Genetic groups of cocoa	Pearson correlation coefficient (R)	P
Catongo	0.66	0.0001
"Criollo"	0.34	0.002
Trinitario	0.51	0.0001
Unknown doubled haploids	0.36	0.04
Amelonado	0.42	0.0001
Upper Amazon doubled haploids	0.01	0.85
Venezuelan	0.18	0.31
Hybrids	0.15	0.03
Upper Amazon	0.20	0.0001
Guianan	0.55	0.0001
Related *Theobroma* species	-	-

Significant differences were also found between genotypes within the groups for both recent and cumulative damage (Table 1). Several Upper Amazon and Guianan clones showed a low level of recent and cumulative mirid damage. These results corroborate those of Sounigo *et al.* (1994) who found, in a similar study, that the least susceptible clones belong to the Upper Amazon group. Indeed, Upper Amazon clones PA120, PA150, NA32, P7, UPA402, UPA413, UPA401 and Amelonado clone IFC2 showed low mirid damage in both studies. On the other hand, clone ICS100 ("Criollo") was among the most susceptible genotypes in both studies. Tables 5 and 6 show, respectively, the most resistant and the most susceptible genotypes within each group for cumulative as well as for recent damage.

The results obtained in this study indicate that susceptibility or resistance of cocoa genotypes to mirids vary from one genotype to another and from one major cocoa group to another. The low level of damage to some of these clones has been attributed to either antixenosis due to lack of attractiveness or antibiosis due to nymphal mortality (Coulibaly 2005). Although the mechanism of resistance has not been clearly investigated, it has been suggested that the behaviour of the clones vis-à-vis mirid attack is controlled by several mechanisms, including colour of the young leaves in the field (Lavabre 1977b), water content of the twigs (Nguyen-Ban 1994), and the presence of phenolic compounds such as flavonol-4 and flavonol-7 (Cros *et al.* 1996).

Table 5. Selected promising cocoa genotypes for field resistance to mirids within different cocoa groups

Genetic groups of cocoa	25% most resistant genotypes within groups (averages scores between brackets)	
	Recent damage	**Cumulative damage**
Catongo	SM3 (0.5), SIC864 (0.5), SIC5 (0.5), IFC376 (1.25)	SM3 (1), SIC864 (1.25), SIC5 (1.25), IFC374 (1.5)
Trinitario	SPEC160-9 (0.5), CC39 (0.25), E1 (C43/2) (0.25), IFC14 (0.25), CC10 (0.25), CF62 (0.25), IFC420(0.25), IFC415 (0.25), CF176 (0.25), SPEC185-4 (0.25), ICS53 (0.25), SPEC138-8 (0.25), IFC422 (0.25), K5 (0.25), SPEC54-2 (0.25), ICS1(0.25), ICS61 (0), ICS9 (0)	SPEC138-8 (0.75), IFC419 (0.75), IFC420 (0.75), IFC14 (0.75) ICS1 (0.75), IFC6 (0.75), IFC7 (0.75), K5 (0.75), CF176 (0.5), DL E26 (0.5), SPEC54-2 (0.5), IFC421 (0.5), DL E31 (0.5), ICS9 (0.5), IFC413 (0.5), IFC415 (0.5), SPEC185-4 (0.5), IFC422 (0.25)
Amelonado	IFC414 (0.25), B11c88 (0.25), A13g9 (0.25), SF123 (0.25), BE3(0.25), B12b181 (0.25), MAT1-9 (0.25), SF121 (0), IFC15 (0), A13d56 (0), IFC2 (0), SF162 (0), SF122 (0), SF142 (0), P4/9 (j114/5) (0), B12b183 (0), PM86 (0), S84 (E104/90)(0), SF201 (0)	BE3 (0.75), B11c88 (0.75), DLF15 (0.75), A13g23 (0.75), A13g14 (0.75), SF161 (0.75), SF162 (0.5), SF131 (0.5), P4/9(j114/5) (0.5), SF223 (0.5), SF201 (0.5), SF171 (0.5), DLF1 (0.5), IFC2 (0.33), SF222 (0.25), SF213 (0.25), PM86 (0.25), B12b183 (0), SF92 (0)
“Criollo”	POR (0), DL OC61 (0), ICS61 (0), DR1XDR38 (1.25), 8P (0)	ICS61 (0.5), ICS48 (0.5), G8 (0.75), ICS40 (0.75), ICS39 (0.75)
Venezuelan	VENC20 (0), VENC4-5 (0.25), VENC4-5 (0.25)	VENC20 (0.25), VENC4-5 (0.5), VENC4-5 (0.75)
Guianan	GU191B (0), GU183K (0), GU221J (0), GU183G (0), GU207G (0), GU226J (0), GU207K (0), GU249B (0), GU209F (0), GU177K (0), GU224R (0), GU265K (0), GU275A (0), GU277G (0), GU290K (0), GU296A (0), GU298B (0), GU318K (0), GU322B (0), GU333K (0), GU226R (0), GU343G (0), GU343F (0), GU343K (0), GU346R (0), GU351F (0), GU179G (0)	GU183K (0), GU226R (0), GU275A (0), GU207K (0), GU298B (0), GU265K (0), GU322B (0), GU333K (0), GU337F (0), GU191B (0), GU343F (0), GU207G (0), GU209F (0.25), GU158K (0.25), GU290K (0.25), GU243B (0.25), GU179G (0.25), GU183G (0.25), GU177K (0.25), GU226J (0.25), GU277G (0.25), GU351F (0.25)
Cloned hybrids	IFC1041 (0), IFC1042 (0), IFC1043 (0), IFC1045 (0), IFC1046 (0), IFC1061 (0), IFC1062 (0), IFC1063 (0), IFC1064 (0), IFC1200 (0), IFC1204 (0), IFC1205 (0), IFC1206 (0), IFC1208 (0), IFC1209 (0), IFC1210 (0)	IFC1063 (0.5), IFC1045 (0.5), IFC1025 (0.5), IFC1046 (0.75), IFC1064 (0.75), IFC1026 (0.75), IFC1038 (0.75), IFC1043 (0.75), IFC1057 (0.75), IFC1039 (0.75), IFC1035 (0.75), IFC1037 (0.75), IFC1047 (0.75), IFC1212(0.75), A1-214-9 (0.75)
Upper Amazon	IFC720 (0), MO81 (0), IFC706 (0), IFC703 (0), IMC31 (0), PA120 (0), P13B (0), IFC682 (0), IFC687 (0), NA79 (0), SCA6 (0), IFC683 (0), P19A (0), AMAZ 15-15 (0), IMC67 (0), SPA9 (0), T79/416 (0), T79/467 (0), IFC678 (0), SCA9 (0), UPA101 (0), UPA106 (0), PA116 (0), IMC78 (0), UPA204 (0), UPA205 (0), UPA216 (0), UPA303, IFC712, P32A (0), UPA402 (0), UPA403 (0), UPA404 (0), IFC726 (0), IFC679 (0), PA150 (0), UPA410 (0), IFC738 (0), IFC698 (0), IFC743 (0), SCA12 (0), UPA512 (0), UPA513 (0), IMC57 (0), IMC6 (0), UPA608 (0), UPA614 (0), UPA615 (0), MO9 (0), MO98 (0), NA2 (0), NA27 (0), NA32 (0), NA58 (0), UPA134 (0), UPA719 (0)	UPA404 (0), UPA218 (0), PA120 (0), IFC683 (0.25), MO81 (0.25), UPA410 (0.25), IFC738 (0.25), UPA205 (0.25), UPA303 (0.25), IMC6 (0.25), UPA608 (0.25), P7 (0.25), UPA719 (0.25), UPA712 (0.25), IFC678 (0.25), IFC698 (0.25), NA27 (0.25), IFC687 (0.25), UPA134 (0.3), IFC703 (0.5), IFC711 (0.5), UPA403 (0.5), IFC682 (0.5), NA79 (0.5), P19A (0.5), IFC718 (0.5), UPA615 (0.5), UPA106 (0.5), UPA413 (0.5), UPA402 (0.5), UPA101 (0.5), NA32 (0.5), UPA713 (0.5), UPA718 (0.5), UPA204 (0.5), AMAZ 15-15 (0.5), UPA701 (0.5), IFC679 (0.5), UPA603 (0.5), MO9 (0.5), UPA401 (0.5), UPA614 (0.5), IFC743 (0.5), UPA407 (0.5), UPA405 (0.5), NA2 (0.5), PA150 (0.5), UPA216 (0.5)

Table 6. Selected cocoa genotypes showing highest susceptibility to mirid damage within genetic groups

Genetic groups of cocoa	10% most susceptible genotypes (averages scores between brackets)	
	Recent damage	Cumulative damage
Catongo	IFC362 (3.75), IFC367 (3.75)	IFC367 (3), IFC368 (2.75)
Trinitario	IFC24 (3), ACT2-11 (3), ACT3-2 (3)	IFC21 (3.25), IFC24 (2.5), IFC22 (2.5)
Amelonado	SF53 (3), A13g44 (2.75), SF52 (2.75)	SF53 (2.25), A13g23 (2.25), PM117 (2.25)
"Criollo"	ICS100 (2.75), POC (2.0)	ICS100 (2.5), POC (2.5)
Venezuelan	VENC4-11 (1), VENC4-14 (1)	VENC4-11 (1.25), VENC4-2 (1.25)
Guianan	GU158G (1.5), GU154B (1.5)	GU158G (1.3), GU125K (1.3)
Cloned hybrids	IFC1213 (1.25), IFC1029 (1.25)	IFC1030 (2.5), IFC1034 (2)
Upper Amazon	UPA705 (1.5), UPA418 (1), UPA710 (1), T63/967 (1)	P19 (1.75), PA121 (1.5), IMC57 (1.5)

Conclusion

The results give a general indication of the level of resistance and/or susceptibility of the major genetic cocoa groups in relation to mirid damage in the field. These results represent useful baseline information for cocoa breeders. If the inheritance of the resistance characters is confirmed through the ongoing studies in Côte d'Ivoire, then the best parents for developing resistant cocoa materials would be several of the Upper Amazon and the Guianan genotypes. The results also suggest that assessment of recent damage is a rapid method for screening for mirid resistance in the field.

Acknowledgement

The authors wish to thank Koné Bakary, Daouda Brété, Kouadio Claude and Nébié Pougda, entomology technicians at the CNRA Research Station in Divo, for their input in carrying out this work. The authors also express their gratitude to the CFC/ICCO/IPGRI project for financing the work.

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Mirid resistance studies in Côte d'Ivoire: assessment of antixenosis, antibiosis and tolerance

K.F. N'Guessan[1], J.A.K. N'Goran[2] and A.B. Eskes[3]

[1] CNRA, BP 808, Divo, Côte d'Ivoire

[2] CNRA, 01 BP 1740 Abidjan 0, Côte d'Ivoire

[3] IPGRI/CIRAD, c/o INIBAP, Parc Scientifique Agropolis II, 34397 Montpellier cedex 5, France

Abstract

A study was conducted to identify sources of mirid resistance in cocoa. Cocoa clones of diverse origins were evaluated for antixenosis, antibiosis and/or tolerance. Antixenosis assessment was based on the number of feeding lesions on twig segments in a choice test. Antibiosis was measured through survival of young mirid nymphs on shoots and on pods, whereas tolerance was assessed through the reaction of the twigs to fresh mirid feeding punctures. Significant differences ($P<0.05$) were found between the genotypes tested with regard to antixenosis, antibiosis and tolerance. Clones T79/501, UPA134, ICS60, UPA409, PA150, IMC57, IFC14, N38, R15, IFC6, IFC15, IFC5, EQX3360-3, LCTEEN, EET59, SCA6, PA107 and Playa Alta 2 were the least attractive to mirids. These clones sustained between 2 and 3 lesions compared to the most attractive ones that sustained between 6 and 8 lesions per twig segment. UPA402, T79/501, T60/887 and IMC67 gave the lowest rate of mirid nymph survival, suggesting that they exhibit antibiosis. Antibiosis on pods appeared correlated with antibiosis on twigs. In the tolerance tests, IMC47, PA150, PA107, SCA6 and C151-61 sustained high numbers of feeding lesions, but showed high rates of recovery from lesions, low levels of dieback and/or an ability to regrow after damage, indicating that they are tolerant to mirid damage. Constraints encountered and the need for further improvement of the test methods are discussed. The results of the resistance test confirmed field observations on cumulative mirid damage for some, but not all, clones.

Introduction

The cocoa mirids, *Sahlbergella singularis* and *Distantiella theobromae*, are the most damaging insect pests of cocoa in Côte d'Ivoire. These insects are also serious pests in other cocoa-producing countries such as Ghana, Nigeria and Cameroon (Lavabre 1970, 1977a; Entwistle 1972). The biology and behaviour of the mirids have been extensively studied (Williams 1953; Taylor 1954; Kay 1961; Gibbs and Pickett 1966; Braudeau 1969; Kumar and Ansari 1974).

Mirids may feed on every part of the plant with the exception of leaves and roots. Both adult and immature stages cause damage through punctures made on vegetative parts or fruiting structures. During feeding, saliva is injected into the wound and this has a marked histolytic effect, probably due to the action of esterases (Williams 1953). On young shoots, the mechanical damage and the effect of the toxic saliva are sufficient to cause their death. On the other hand, on hardened twigs and stems, the mechanical effect is less important. However, subsequent invasion of the wounds by a pathogenic fungus, *Calonectria rigidiuscula*, has been reported (Crowdy 1947). These attacks result in cankering or bark roughening, destruction of the flower cushions and a severe dieback of twigs and branches.

On fully-grown pods, the feeding sites are marked by black spots of dead tissue, but maturation may continue. However, on young pods or cherelles, a high number of feeding punctures may cause distortion during growth or even death of the fruit. Yield losses attributed to mirid damage alone have been estimated at 30-40% (Lavabre 1977a).

The principal means of mirid control has been, for many years, the application of chemical insecticides on the basis of a calendar spray schedule (Lavabre 1960; Decazy and Essono 1979; Coulibaly *et al.* 1996). The adoption of chemical control has been difficult for many farmers because of the high cost of the chemicals and of the spraying equipment, and low availability of water for the preparation of the spray mixture. In addition, there are several other problems related to chemical use, including environmental contamination, disruption of biodiversity, effect on non-target organisms and health concerns.

In Côte d'Ivoire research has been oriented towards the development of alternative methods. The development and use of mirid-resistant cocoa varieties is one of the alternatives to chemical control. Mirid resistance studies in cocoa have been undertaken by several researchers including Bruneau de Miré and Lotodé (1974), Decazy and Lotodé (1975), Decazy and Coulibaly (1982), Nguyen-Ban (1994) and Sounigo *et al.* (1994). However, these studies have mostly concentrated on assessment of field damage and progress so far has been limited. No work has yet clearly mentioned the mechanisms of mirid resistance in cocoa. The current study was designed to evaluate selected cocoa genotypes for resistance to mirids, with special attention to antixenosis, antibiosis and tolerance.

Materials and methods

The study was conducted in 2000-2003 at the research station of the Centre National de Recherches Agronomiques (CNRA) in Divo, Côte d'Ivoire, within the framework of CFC/ICCO/IPGRI project. The genotypes tested were from various origins. Some genotypes were clones used as parents in the reciprocal recurrent selection (RRS) programme in Côte d'Ivoire. Another group was made up of "international clones", which were introduced from the quarantine facilities of the University of Reading (UK) and from the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD, France). Others were selected local clones either used as parents in the breeding programme or planted in the main germplasm collection in Divo. The genotypes were evaluated separately for antixenosis, antibiosis and/or tolerance to mirids (*S. singularis*). The methods applied follow the recommended Working Procedures for the CFC/ICCO/IPGRI project (Eskes *et al*. 2000), with small modifications described hereafter.

Evaluation of antixenosis

This study was conducted with the objective of identifying cocoa genotypes showing no or a low level of attractiveness to *S. singularis*. The material tested included RRS parents and the international clones.

Fifty RRS parental clones were evaluated in two separate groups of 25. One group was made up of the Lower Amazon (LA) and Trinitario parents, and the second of Upper Amazon (UA) parents. The experimental design was an incomplete block with 6 replicates and 30 blocks. Such a design allows the comparison of each genotype with the others within the same experimental set-up (Cochran and Cox 1957). Twenty-four international clones, and one locally selected clone (C15-161), were evaluated in a similar manner, using clone T79/501 as a resistant control. Indeed, clone T79/501 has previously been shown to be promising for mirid resistance on the basis of damage in the field (Sounigo *et al*. 1994).

Healthy green twigs of young flushes obtained from the field were brought to the laboratory and cut into 6-cm sections. Five fragments of twigs with the same diameter and representing five different cocoa genotypes were placed end-to-end in 30 large Petri dishes (16-cm diameter x 2-cm height), according to the statistical design. One 4th instar mirid nymph that had been starved for 24 hours was placed in each Petri dish. Mirid nymphs were collected from the field the day before using them in the experiment. The insects were

allowed to feed for 24 hours and the feeding lesions on the fragments of twigs were counted for each genotype in order to assess attractiveness of the different genotypes. Data were analyzed using the GLM procedure of SAS (SAS Institute 1996).

Evaluation of antibiosis

This study was carried out with the objective of assessing the survival and the development of cocoa mirids on selected cocoa genotypes. The material evaluated included 16 selected local clones. These clones were tested on both twigs and pods.

A no-choice test was conducted to assess mirid survival and development. The design was a completely randomized design with 5 replications for the test on twigs and 8 replications for the test on pods. Healthy green flush or chupon twigs were labelled in the field or in a budwood garden. Second instar mirid nymphs were obtained from a field collection. A nylon mesh sleeve cage (170-cm height x 30-cm diameter) was used to confine two second-instar mirid nymphs on a twig or on a chupon. Confinement of mirids on twigs in this experiment took place in July and August 2000. Both ends of the cage were securely tied to prevent the insects from escaping. The shoots were kept in their natural position by tying the upper part of the sleeve cage to a stake and the lower part to the twigs. Glue was applied on the twigs or chupons and around the stake to prevent any ants from attacking the mirids in the cages. In addition, any other shoots in contact with the cages were cut off. On susceptible genotypes, the twigs sometimes dried up from feeding punctures before the insects completed full development into adults. In such situations the nymph was moved to a new twig on the same tree to continue observations on its development. For the test on pods, five second-instar nymphs were confined on mature green pods. Tests on pods were conducted from September to November 2000. Usually more than five mirid nymphs can complete development on one pod. Here again precautions were taken to prevent ants from attacking the mirids.

The nymphs were allowed to feed on the shoots or pods until they died or became adults. For the shoots, a replicate was a tree with three cages, and for the pods a replicate was a tree with two cages. The mortality of the nymphs was recorded every two days, and at the end of the experiment, the rate of mirid survival was determined for each genotype.

Evaluation of tolerance

The study was conducted on 17 international clones with the objective of identifying cocoa genotypes capable of withstanding or recovering from mirid damage. The experiment was based on the assessment of the level of dieback of twigs and recovery from damage in response to mirid feeding punctures. The experimental design was complete randomization of 4 replicates. A sleeve cage (170-cm height x 30-cm diameter) made with mosquito screen nylon mesh was used to confine one 4th instar mirid nymph on a single semi-hardened healthy twig for 48 hours. The insect was then removed and the initial number of feeding lesions was counted. The twig was inspected for dieback and regrowth weekly for the first month and then monthly for the next three months. Confinement of mirids on twigs in this experiment started at the end of October 2002 and ended before the dry season started, by the end of November 2002. For the assessment of the degree of dieback, a score of 0 to 4 was given to the shoot, with 0 corresponding to no dieback symptoms, 1 to 25% of the leaves showing dieback, 2 to 50% of the leaves showing dieback, 3 to 75% of the leaves showing dieback and 4 corresponding to shoots with all the leaves showing dieback.

Results and discussion

Antixenosis

Significant differences were found between the Lower Amazon RRS parents (DF=24; F=2.33; P=0.002) and between the Upper Amazon RRS parents (DF=24; F=3.42; P=0.0001) with regard to the number of lesions. However, there was a relatively large overlapping of groups of significance according to the Waller-Duncan test. Clones T79/501, UPA134, ICS60, UPA409, PA150, IMC57 (UA parents) and IFC14, N38, R15, IFC6, IFC15, IFC5 (LA parents) appeared to be the least attractive (Table 1). These clones showed between 2 and 3.5 lesions compared to the most attractive ones which showed between 6 and 8 lesions.

Table 1. Attractiveness of selected Upper Amazon and Lower Amazon clones to the cocoa mirid, *S. singularis*

Upper Amazon parents		
Clones	**Lesions**	**Means grouping**
P19A	7.6	a
POR	7.3	ab
MO98	6.8	abc
NA32	6.8	abc
P7	6.7	abc
UPA413	6.7	abc
T85/799	6.1	abcd
MO81	6.0	abcd
PA4	5.7	abcde
UPA401	5.4	abcdef
NA58	5.0	bcdefg
T60/887	4.6	cdefgh
DLOC61	4.5	cdefgh
SCA6	4.4	cdefgh
IMC6	4.0	defgh
UPA402	4.0	defgh
ICS39	4.0	defgh
IMC67	3.9	defgh
G8	3.9	defgh
IMC57	3.4	efgh
PA150	3.1	fgh
UPA409	3.1	fgh
ICS60	2.7	gh
UPA134	2.6	h
T79/501	2.5	h

Lower Amazon parents		
Clones	**Lesions**	**Means grouping**
UF667	8.0	a
IFC371	7.7	ab
SNK12	7.6	ab
ACU85	7.4	abc
MAT1-9	6.0	abcd
ICS84	5.7	abcd
W41	5.5	abcd
WA40	5.5	abcd
ICS6	5.1	abcd
UF676	5.0	abcd
ICS89	4.5	abcd
IFC11	4.4	abcd
GS29	4.3	abcd
MAT1-6	4.1	abcd
ICS95	4.0	abcd
ICS46	4.0	abcd
CC10	3.9	bcd
IFC29	3.5	bcd
IFC8	3.5	bcd
IFC5	3.3	bcd
IFC15	3.2	bcd
IFC6	3.1	cd
R15	3.0	cd
N38	2.9	cd
IFC14	2.0	d

Means followed by the same letters are not significantly different (P>0.5, Waller-Duncan K-ratio T-test).

The number of feeding lesions also varied significantly (DF=24; F=3.81; P=0.0001) among the international clones. The clones EQX3360-3, LCTEEN46, EET59, SCA6, PA107 and Playa Alta 2 were the most promising with regard to antixenosis (Table 2). Indeed, these clones had a lower number of lesions (2 to 3) than the others (4.5 to 6).

Table 2. Attractiveness of the international clones to the cocoa mirid, *S. singularis*

Clones	Lesions	Means grouping
AMAZ5-2	5.9	a
AMAZ15-15	4.9	ab
SPEC54-1	4.7	abc
C15-161	4.5	abcd
GU255V	4.5	abcd
Catie1000	4.4	abcd
MAN15-2	4.2	bcde
T85/799	4.1	bcde
Mocorongo	4.1	bcdef
PA120	3.9	bcdefg
T79/501	3.8	bcdefg
BE10	3.7	bcdefg
P7	3.6	bcdefgh
ICS1	3.3	bcdefgh
MXC67	3.1	cdefgh
IMC47	3.1	cdefgh
VENC4-4	3.0	defgh
IFC5	3.1	defgh
EQX3360-3	2.9	defgh
PA150	2.7	efgh
EET59	2.6	efgh
LCTEEN46	2.6	efgh
PA107	2.4	fgh
SCA6	2.2	gh
Playa Alta 2	2.0	h

Means followed by the same letters are not significantly different (P>0.5, Waller-Duncan K-ratio T-test).

Antixenosis denotes the presence of morphological or chemical plant factors that alter the insect's behaviour, resulting in the insect moving away and selecting a different host plant. When different cocoa genotypes are exposed to cocoa mirids, there are often differences in the level of preference of the mirids for these genotypes. This is usually expressed by the differences in the number of feeding lesions in the laboratory and the level of dieback in the canopy in the field. In the present study, clones T79/501, UPA134, ICS60, UPA409, PA150 and IMC57 (UA parents), clones IFC14, N38, R15, IFC6, IFC15 and IFC5 (LA parents), and clones EQX3360-3, LCTEEN46, EET59, SCA6, PA107 and Playa Alta 2 (international clones) showed lower numbers of lesions compared to other clones. This suggests that these clones show some level of antixenosis. These results agree partly with those obtained by Sounigo *et al*. (1994), who found T79/501 and PA150 also to be promising for mirid resistance based on cumulative field damage observed in clone trials. In addition, other studies indicated that UPA134 was less attacked by mirids in the field compared to neighbouring clones (Lavabre 1977b).

Antibiosis

Significant differences were found between clones with regard to mirid survival on twigs (DF=15; F=2.4; P=0.01) and on pods (DF=15; F=3.5; P=0.0001). On twigs, clones with the lowest rate of mirid survival were UPA402, T79/501, T60/887 and IMC67 (Table 3). On the other hand, IMC67, T79/501, UPA402 and GS36 showed the lowest rate of survival on pods (Table 3). Correlation between the antibiosis test on pods and on twigs was positive and highly significant (r=0.90), indicating that the results from both tests were consistent. When considering ranking for both traits, the three most resistant clones were UPA402, T79/501 and IMC67 and the three most susceptible clones SNK12, P7 and NA32.

The low rate of survival of mirid nymphs shown on clones UPA402, T79/501, T60/887, ICS95, IMC67 and GS36 suggests that these clones show some level of antibiosis. Three of these clones (UPA402, T79/501 and T60/887) also showed low cumulative mirid damage in field

observations; however, this was not the case for GS36 (Sounigo *et al*. 1994). In addition clone T79/501 also showed low-to-intermediate attractiveness (Tables 1 and 2). These results also corroborate in part those of Decazy and Coulibaly (1982) who found that UPA402 is associated with a low number of mirids in the field. Although the causes of antibiosis have not been investigated in this study, previous works on other crops have attributed antibiosis to either the presence of toxins or growth inhibitors in the plant (Smith 1989).

Table 3. Survival of mirid nymphs on cocoa twigs and pods of selected cocoa genotypes

Survival rate on twigs		
Clone	**Survival rate**	**Means grouping**
P7	100.00	a
PA150	91.67	ab
SNK12	91.67	ab
SCA6	88.89	abc
GS36	88.89	abc
IFC5	83.34	abc
NA32	77.78	abc
IFC371	75.00	abc
UF667	73.34	abc
ICS39	66.67	abcd
T85/799	66.67	abcd
ICS95	66.67	abcd
IMC67	66.67	abcd
T60/887	58.34	bcd
T79/501	55.56	cd
UPA402	33.33	d

Survival rate on pods		
Clone	**Survival rate**	**Means grouping**
SNK12	92.50	a
NA32	92.00	a
T60/887	90.00	a
UF667	87.50	ab
IFC371	86.67	ab
ICS95	83.33	ab
P7	82.86	ab
ICS39	82.50	ab
PA150	80.00	abc
SCA6	80.00	abc
IFC5	80.00	abc
T85/799	70.00	bcd
GS36	62.86	cd
UPA402	62.86	cd
T79/501	62.86	cd
IMC67	60.00	d

Means followed by the same letter are not significantly different (P>0.5, Waller Duncan K ratio T test).

Tolerance

The results showed high variability among the clones tested with regard to the number of mirid feeding lesions (DF=16; F=3.8; P=0.0001), the level of the resulting dieback (DF=16; F=2.2; P=0.005), the rate of recovery from lesions (DF=16; F=2.5; P=0.001) and the level of regrowth (DF=16; F=14.8; P=0.0001). Clones IMC47 and PA150 were identified as potentially tolerant clones. These clones showed a high number of lesions, but showed a high rate of recovery from lesions and a low level of dieback (Figs. 1, 2, 3). Clones SCA6, PA107 and C151-61 may also be considered as promising because they have a good ability to regrow after damage (Fig. 4).

Plant tolerance to insects is characterized by the ability of the host plant to grow or yield normally while supporting an insect pest population that usually causes severe damage to a susceptible host (Painter 1951). Thus, tolerance is often determined by comparing the production of biomass (yield) in insect-infested and non-infested plants of the same cultivar (Smith 1989). In the present study, determination of cocoa tolerance to the cocoa mirids was based on assessment of plant reaction to attacks. Clones IMC47, PA150 and PA107 sustained a high number of lesions, but showed a high rate of recovery from lesions and a low level of dieback. Such clones with high recovery rate from lesions will be able to recover faster from damage than clones with low tolerance. As a result, they would be able to maintain more flower cushions and yield better than clones with low tolerance.

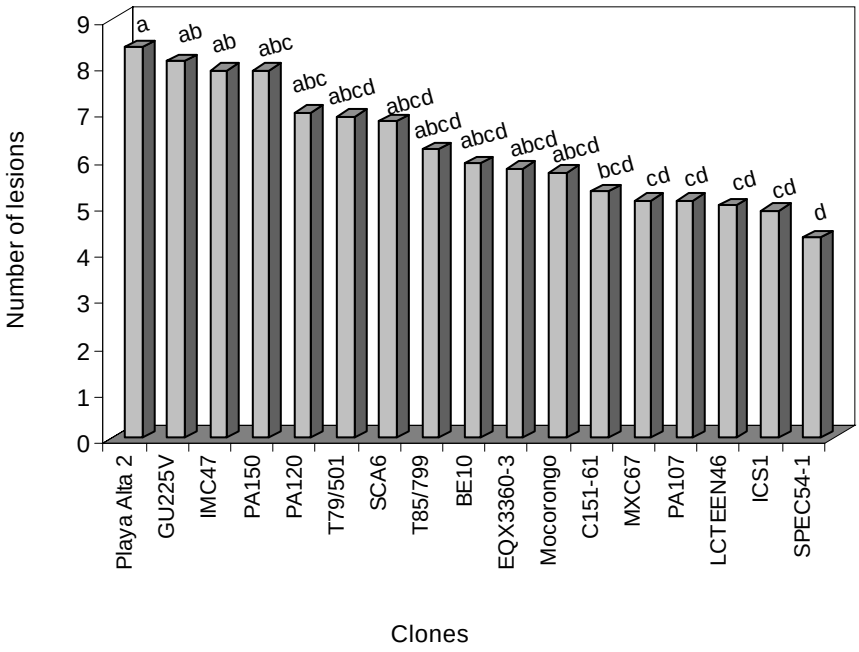

Fig. 1. Numbers of mirid feeding lesions recorded from forced feeding on twigs of selected international clones.

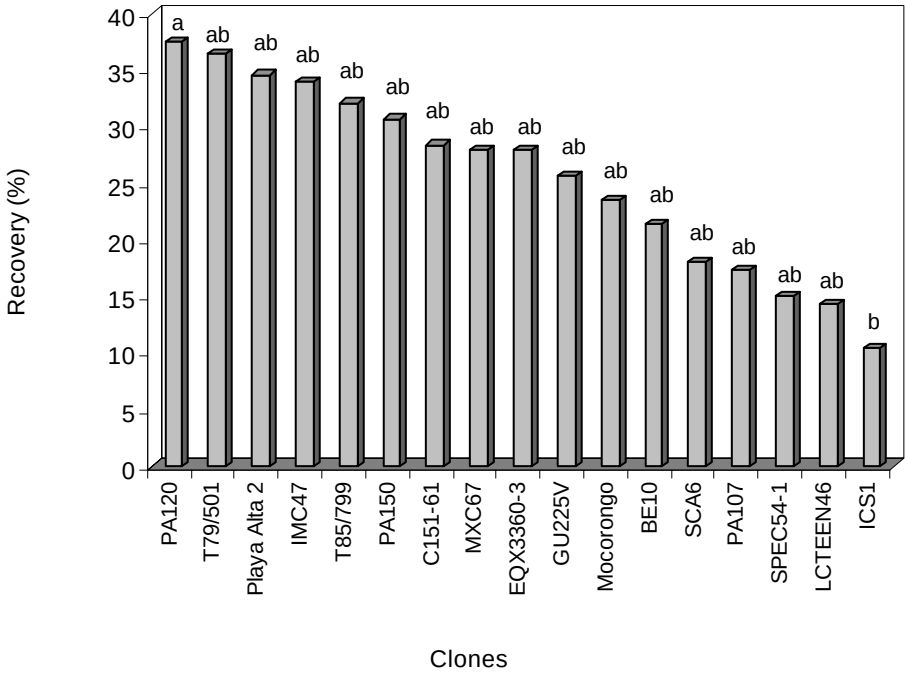

Fig. 2. Recovery from fresh mirid feeding lesions of selected international clones.

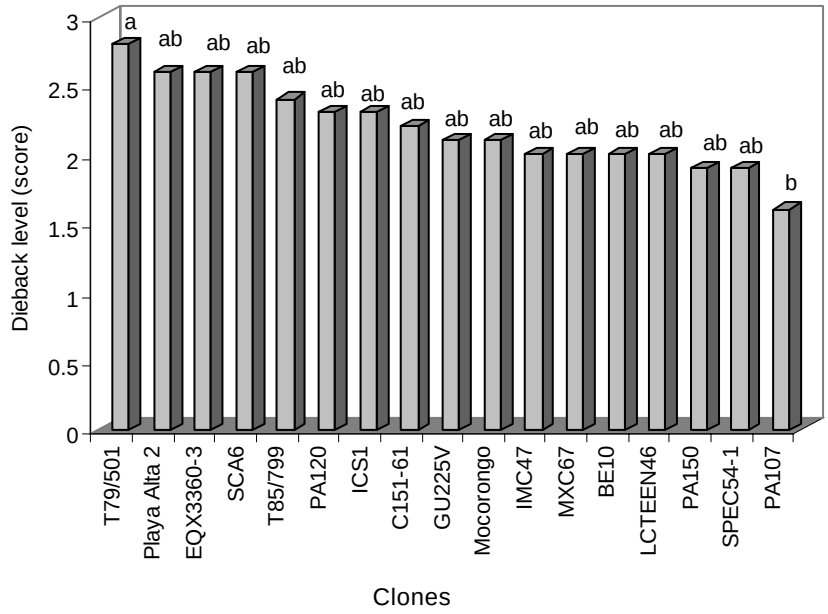

Fig. 3. Dieback from mirid feeding lesions on selected international clones.

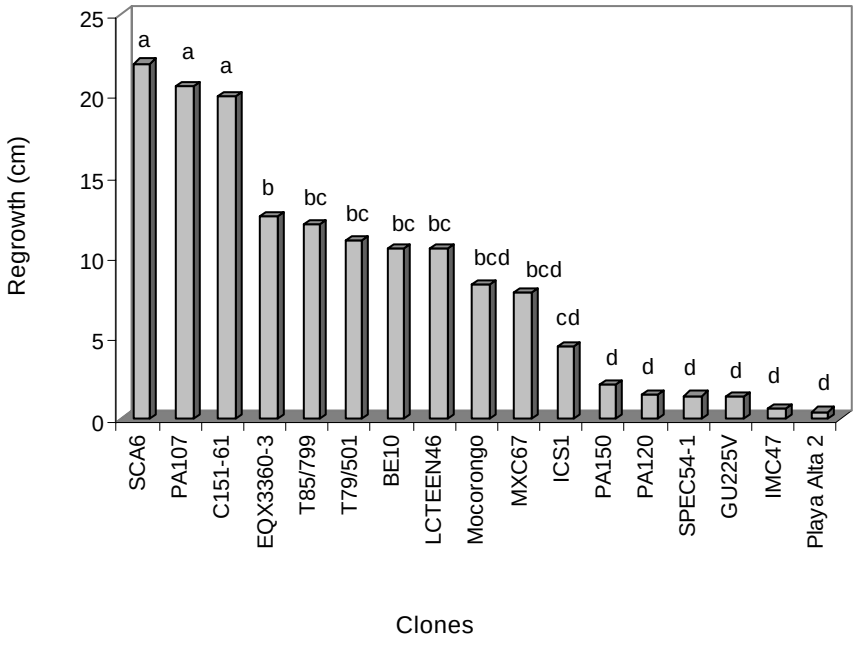

Fig. 4. Regrowth of shoots damaged by mirid feeding lesions of selected international clones.

Conclusions and recommendations

Significant differences were found between the genotypes tested with regard to antixenosis, antibiosis and tolerance, suggesting that some genotypes have at least low-to-moderate level of mirid resistance/tolerance. These results can be considered as a baseline for future studies regarding cocoa resistance to mirids. Indeed, a number of problems have been encountered while conducting the different experiments. These problems include the fragility of mirids making manipulation sometimes difficult, the problem of availability of mirids for tests because a good rearing technique is lacking, mirid predation by ants in the sleeve cages during field tests, and the need to wait for flushes for mirid resistance tests coupled with unequal flushing among different genotypes. Because of the many constraints related to performing some of the tests, it remains necessary to pursue the improvement of some of the screening tests. These improvements will need to consider the plant material used, the stages of the insect used in the tests, and the parameters to be measured.

The fungi that might be involved in the dieback process need to be isolated, identified and their pathogenicity evaluated. Once demonstrated, a more appropriate early screening test for tolerance might be developed, based on the reaction of the plant to the fungi associated with dieback. In addition, another type of investigation should consider the effect of the mirids and more precisely the impact of their saliva on the plant tissue. This investigation should include the determination of the chemical composition of the saliva and the effect of each substance on the dieback process. During such a study, comparison of *Salhbergella singularis* and *Distantiella theobromae* will be necessary because attacks of *D. theobromae* cause faster dieback than those of *S. singularis.*

Acknowledgements

The authors wish to express their gratitude to the CFC/ICCO/IPGRI project for financing this study.

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Field performance of some local and international clones of cocoa against infestation by mirids

R. Adu-Acheampong, B. Padi, J.B. Ackonor, Y. Adu-Ampomah and I.Y. Opoku
CRIG, PO Box 8, Tafo-Akim, Ghana

Abstract
The two most important insect pests of cocoa (*Theobroma cacao* L.) in West Africa are *Sahlbergella singularis* and *Distantiella theobromae*. They cause yield loss of up to 30% and are controlled primarily by the use of conventional insecticides. A minimum of four insecticide applications at 28-day intervals may be required on mature cocoa each year. However, environmental pollution and issues of residues in beans and chocolate call for more environmentally friendly and sustainable control strategies. The development of mirid-resistant cocoa genotypes offers an opportunity to withstand the pest menace. This study reports on findings of field studies to evaluate 25 local and international clones for resistance/tolerance to mirids. The mirid population was lowest on IFC5, followed by Mocorongo, LCTEEN37F and SPEC54-1. EET59 had the highest mirid population followed by T85/799 and T79/501. In terms of expression of damage symptoms, T85/799 was the most tolerant, followed by LAF1 and PA107. The most susceptible clones were EET59 followed by LCTEEN37F, IFC5 and EQX78. Genotypes that remain vigorous in the presence of high mirid populations (PA107, T85/799, T79/501), and those with low mirid populations and damage under natural field conditions (e.g. Mocorongo) should be of interest. These clones have the potential of being included in mirid resistance breeding programmes for the improvement of cocoa. It is important to evaluate their agronomic characteristics in order to possibly recommend them for smallholder farmers.

Introduction
Cocoa is an important cash crop in many West African countries (Acquaah 1999). In Ghana, the crop is grown on over 2 million ha mainly by small-scale farmers and over 730 000 t were produced in the 2003-2004 season.

Sahlbergella singularis and *Distantiella theobromae* are undoubtedly the two most important insect pests of cocoa in West Africa (Entwistle 1972) and extensive feeding on cherelles and branches can substantially reduce bean yield. Young cocoa (i.e. trees under 3 years) are particularly susceptible as the time to come to fruit bearing may be delayed by several years.

Mirid control relies primarily on synthetic insecticides and a minimum of four insecticide applications at 28-day intervals may be required on mature cocoa each year. Recently, some relief from mirid infestations and black pod (*Phytophthora* pod rot or Ppr) infection have been achieved in Ghana by the National Cocoa Diseases and Pests Control Programme (CODAPEC). However, chemical control is still less than desired and other strategies such as host plant resistance, that rely less on traditional insecticides for pest control, would be useful for long-term management. Available evidence (Adomako and Ackonor 2003) indicate the potential for exploitation of plant resistance/tolerance.

Several studies have been conducted on breeding for resistance against insects, but most of them have focused on cotton and tomato (Barten *et al.* 1994), vegetables (Stoner 1970; Brown *et al.* 1995) and wheat (Obanni *et al.* 1989).

Materials and methods

Twenty-five cocoa clones were evaluated for their resistance to mirid attack at Tafo (Ghana) from 2002 to 2004. This location has a humid, mild climate, with an annual rainfall of ca. 1600 mm. The clones were planted at the same time and the number of stands of the different clones in the plot ranged from 3 to 12. At approximately 28-day intervals, the number of mirids present on each plant (up to 2 m above ground level) was recorded, and the population per plant used as an index of preference. Visual damage rating of the tree canopies, based on a 9-point scale (from zero being without dieback to 9 being completely damaged by dieback) was recorded.

Results and discussion

The study has demonstrated distinct differences in the level of resistance among clones based on plant damage rating as well as differential attractiveness of clones to the insects (Table 1). There is, therefore, a good reason to believe that resistance to mirid attack could successfully be evaluated in the field. Among the 25 clones, 11 were tolerant to mirid attack: PA120, Mocorongo, PA107, SCA6, LAF1, T85/799, PA150, ICS1, MO20, GU225V and T79/501. These clones had comparatively lower mean damage scores. Twelve other clones, including EQX3360-3, UF676, ICS43, AMAZ15-15, LCTEEN37-I and MAN15-2, were moderately tolerant. EET59 and IMC47 were the most susceptible clones to mirid attack. This is in agreement with earlier laboratory and cage experiments in which IMC47 recorded the highest number of feeding lesions (punctures) per twig. Fig. 1 shows the severe damage caused by mirids on clone EET59.

Table 1. Mean mirid density and plant damage scores on 25 clones in the field

Clone	Mean mirid density (per plant)	Mean plant damage score
EET59	17	4.6
T79/501	17	1.0
T85/799	13	0.1
PA107	9	0.3
MO20	9	0.9
UF676	8	1.8
AMAZ15-15	7	1.3
LCTEEN37-I	6	1.7
IMC47	6	4.0
MAN15-2	6	1.2
PA150	5	0.6
BE10	5	1.5
LAF1	5	0.2
VENC4-4	4	2.0
PA120	3	0.6
ICS43	3	1.8
SCA6	2	0.6
GU255V	1	0.8
EQX78	1	2.5
EQX3360-3	1	1.7
ICS1	1	1.0
LCTEEN37F	1	2.7
Mocorongo	1	0.6
SPEC54-1	0	1.9
IFC5	17	2.4

Fig. 1. Progeny EET59 showing "blast". Plants with damage as severe as this do not produce any yield; mirids are the main pests behind this damage.

The inherent genetic differences among the clones were clearly displayed in their growth patterns. In screening cocoa germplasm for resistance to mirids, those that remain vigorous in the presence of high mirid populations (PA107, T85/799 and T79/501), and those with low mirid populations and damage under natural field conditions (e.g. Mocorongo) should be of interest. The tolerant clones have the potential of being included in mirid resistance breeding programmes for the improvement of cocoa and if the observed differences between the clones could be verified to be consistent, one could possibly do breeding for mirid resistance by crossing clones with low number of mirids with clones with low damage score. It will then be imperative to assess their agronomic characteristics in order to recommend the best of them to smallholder farmers. These comments are only based on preliminary observations made on the germplasm collection, and the materials would need detailed verification in the international clones trial with due replication of the clones. The susceptible clones, including EET59, could only be cultivated successfully with the protection of conventional insecticides.

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VSD RESISTANCE STUDIES

Evaluation of cocoa resistance to vascular streak dieback in Malaysia

M.J. Ahmad Kamil, L. Kelvin, S. Sapiyah, M.T. Lee, S. Shari Fuddin, L. Albert, A. Francis and C.L. Bong

Malaysian Cocoa Board (MCB), Locked bag 211, 88999 Kota Kinabalu, Sabah, Malaysia

Abstract

The CFC/ICCCO/IPGRI international cocoa project fits well in the Malaysian Cocoa Board (MCB) research and development programme with regard to the development of high-yielding and disease-resistant planting materials. In Malaysia, two methods are used for evaluation or screening for resistance to vascular streak dieback (VSD): dual *in vitro* culture and field observation. The *in vitro* method is used for preliminary screening of potentially resistant clonal and hybrid materials in the laboratory. This method is non-destructive, fast and cheap, and screening can be done on a large scale. The field method is based on visual scoring of VSD infection under normal planting conditions. Results are shown on one-year field evaluation data carried out in the MCB trials that were supported by the project, and also data on *in vitro* screening of some of the project materials. The preliminary field results suggest good levels of VSD resistance in the population breeding trials and in the Local Clone Observation Plot (LCOP). Differences between clones in the International Clone Trial (ICT) were hardly significant. The coefficient of correlation was negative, as expected, but not significant. Some of the clones that had low VSD scores in the *in vitro* method did not necessarily show low scores in the field screening. The difference in the resistance between the *in vitro* and field evaluation among the clones might be due to variation in plant development and shade intensity. More years of observation and data collecting are probably required to conclude on the correlation between *in vitro* and field evaluation. However, the *in vitro* inoculation is considered useful for early screening to identify clones and hybrids with potentially higher resistance to VSD. Continued field evaluation of the project materials will be carried out in the second phase of the project (2004-2009).

Introduction

Vascular streak dieback (VSD), caused by the fungus *Oncobasidium theobromae*, is a disease of high economic importance in Malaysia. The disease affects both the young seedlings and mature plants. The fungus infects the vascular tissues of the tree, which causes the leaves to drop and new shoots to die. A distinctive yellowing of the leaves is also observed. The vascular tissues become discoloured, forming brown streaks at the cambium layer under the bark. In Malaysia, the average yield loss due to VSD ranges between 10 and 15%. Without effective control, VSD can be a problematic disease in the nursery and in field plantations. VSD can be effectively controlled by the use of fungicides and pruning of the infected branches. However, continuous fungicide spraying can be costly. Resistant planting materials are preferred as a more effective way to manage the disease.

Past research undertaken by the various research agencies in Malaysia resulted in the selection of VSD-resistant clones (e.g. PBC123 and KKM25) and hybrids (e.g. UITI x NA33 and PA138 x SCA9). However, the number and choices of the VSD-resistant material are limited. This paper presents the result of the VSD assessment using the *in vitro* and field evaluation of the CFC/ICCO/IPGRI project materials evaluated by the Malaysian Cocoa Board (MCB).

Current practices in the management of VSD disease

Research on the various aspects of VSD management conducted in Malaysia since the 1960s has been reviewed by Tay and Bong (1989, 1992, 1995) and Musa and Bong (1990). Currently, several methods are used, as follows.

Physical protection methods

Physical protection is commonly used in the cocoa nurseries. Clear polyethylene plastic sheets are used as roofing material to avoid wetting the foliage of seedlings and VSD infection. It is cost-effective for seedling preparation for large-scale planting. In some cases, it may be needed to protect also newly transplanted seedlings by partially covering them with plastic sheets or with used fertilizer bags.

Chemical control

Fungicides are more commonly used to control VSD in the nursery. Flutriafol and triadimenol were reported to be effective in controlling VSD in both young and matured cocoa (Bong and Seow 1989; Lam *et al*. 1992). The fungicides can be applied as a foliar spray or soil drenching.

Cultural practices

Sanitation pruning is a routine general practice in cocoa holdings to discard infected plant tissues. It is a routine practice to shape the canopy for better light penetration and to facilitate pest and disease control. Fertilizer input has also been found to be effective in reducing VSD disease severity (Tay *et al*. 1989).

Use of resistant planting material

The use of resistant planting materials is widely practiced in Malaysia. A number of locally selected clones were identified to be resistant to VSD, e.g. PBC123, KKM22, DESA1, QH22, QH37, QH1003 and C57. In addition, several commercial hybrid varieties that were proven to be resistant, such as UIT1 x NA33 and PA138 x SCA9, have been used as control hybrids in most progeny trials carried out by MCB.

Integrated VSD management

An integrated management approach can be effective in controlling VSD. A combination of shade management, fertilizer input, resistant planting materials and frequent pruning could reduce effectively VSD severity and increase productivity.

Evaluation of resistance to VSD

In vitro screening

- **Method used**

This screening method was originally described by Bong and Puad (1995) and modified by Bong (2000) as part of the "Working Procedures for Cocoa Germplasm Evaluation and Selection" for the CFC/ICCO/IPGRI project. The screening method starts with the preparation of cocoa calli that are initiated from petioles and maintained on Murashige and Skoog (MS) medium supplemented with kinetin and indole acetic acid (IAA) at 5 and 10 mg/l respectively. The calli cultures were incubated in the dark at a temperature ranging from 25°C to 28°C. The calli were sub-cultured in universal bottles each containing 10 ml of sterilized medium after 4 to 5 weeks. Once enough calli were obtained (after about 5 weeks), they were divided into pieces of about 2 x 5 x 5 mm that were used in the resistance

screening. Preparation of the inoculum of *Oncobasidium theobromae* was done after fresh isolation of the mycelium from infected twigs on 1% water-agar. Mycelia discs of 5-mm diameter, taken from the margin of the colonies, were obtained from 10-day-old cultures. For screening of resistance in dual cultures, the same medium and culture conditions were used.

Conical flasks of 100 ml containing 50 ml medium were used. One piece of the callus was placed into each flask. Each callus was inoculated with one mycelium disc placed besides the callus on the culture medium (dual culture). The mycelium disc was also placed beside and on top of the callus. A single culture of callus, without inoculum, was used as control. The cultures were incubated for 4 to 6 weeks, after which the calli were harvested. Mycelium on the surface of the callus was removed and the dry weights of calli were recorded. Mycelia scores were obtained by visual estimation of the intensity of mycelium growth on the calli and on the growth medium which provided an indication on the extent of growth of the fungus in the dual culture. The range or average value of mycelial scores gave an indication of the vigour of the pathogen isolate used in the screening of the particular batch.

The formula used to derive the resistance index (R-index) was:

$$R = (I) / (MH)$$

Where:

I = average dry weight of inoculated calli
M = average mycelial score of inoculated calli
H = average dry weight of un-inoculated calli

A high R-index indicates a high relative resistance (R value is not absolute). Any R-indexes higher than those of the controls are considered resistant.

- **Results**

One hundred clones were screened in three batches (5B, 8B and 9B) depending on availability of callus tissue of the clones (Table 1). The resistant clone NA33 was used to compare the resistance of the clones within each batch.

In Batch 5B, TG146, QH1560, P12, UF676, SDS19, QH968, QH496, UP8, QH1176, UP2, K82, TG266, QH37, SDS52 and KKM25 had a higher R-index than NA33. Among these clones, TG266, QH37, SDS52 and KKM25 had an R-index threefold higher than NA33, which indicated that they were the most tolerant clones in Batch 5B. QH37 was also reported to be resistant to VSD in the field (Chong 2000) while TG266 and SDS52 were observed to exhibit a high level of VSD resistance in one of the MCB clone trial. KKM25 is known to be resistant to VSD.

Twenty-three of the clones in Batch 8B had an R-index lower than that of NA33, including PBC123, a widely resistant clone planted in Malaysia. Clones with higher R-indexes than NA33 were QH968, BAL140, PBC159, MAN15-2 and QH1003. QH1003 exhibited the highest R-index. Chong (2000) also reported that this clone was one of the most resistant in their collection.

Results in Batch 9B showed that E93 (DESA), LS8, PBC233, QH 326, PA150, EET158, DESA101, DESA105, DESA102, QH1345, QH1000, BAL263, LAF1, SDS56, QH1346, QH1075, QH1126 and MXC67 had higher resistance indexes than NA33. QH1003 and QH1346 were also reported to be resistant to VSD in the field evaluation carried out by the Department of Agriculture, Sabah (Chong 2000). MXC67 also had a low level of VSD infection in the field evaluation of the ICT conducted by MCB.

The results of this study suggest that the *in vitro* method can be used as preliminary screening for resistance to VSD.

Table 1. Results from *in vitro* screening of local and international clones for resistance to VSD. Calli from petiole tissue were used for batches 5B, 8B and 9B (resistant check clone in bold)

Batch 5B			Batch 8B			Batch 9B		
Clone	**M**	**R**	**Clone**	**M**	**R**	**Clone**	**M**	**R**
FP3	4.3	0.011	TG149	2.4	0.029	SIC5	4.0	0.015
QH670	2.7	0.012	KKM1	2.0	0.036	SCA6	4.0	0.017
EQX3360-3	4.0	0.015	QH1135	3.0	0.037	TG157	5.0	0.018
Mocorongo	5.2	0.017	QH1247	2.0	0.041	QH1287	4.0	0.044
KKM2	4.0	0.017	BAL263	2.5	0.047	QH185	4.0	0.049
QH731	4.3	0.025	P7	2.2	0.051	QH441	4.7	0.052
Playa Alta 2	4.0	0.026	TG149	3.3	0.053	DESA1	6.3	0.065
PA107	4.3	0.026	QH1285	2.7	0.058	QH441(B11)	4.0	0.069
PBC236	3.7	0.027	KKM22	3.3	0.062	TG137(A48)	3.3	0.069
SIAL339	3.2	0.031	DAS51	4.0	0.065	ICS43	1.3	0.099
PBC179	4.7	0.036	PBC123	2.0	0.072	QH1213	6.3	0.105
FP5	4.3	0.036	QH794	2.0	0.076	SDS4	4.7	0.110
NA33	**4.4**	**0.040**	QH1346	1.8	0.073	**NA33**	**5.7**	**0.126**
TG146	4.0	0.044	QH1345	1.3	0.081	BR25	3.0	0.132
QH1560	3.0	0.044	TG148	2.7	0.094	FP2	4.0	0.175
P12	3.0	0.045	P7A	2.2	0.095	QH1287	1.0	0.193
UF676	4.7	0.046	SDS62	3.3	0.098	FP1	2.2	0.203
SDS19	4.0	0.047	AMZ15-15	1.4	0.107	QH938	3.3	0.218
QH968	4.7	0.047	BAL300	2.7	0.118	KKM25	2.4	0.224
QH496	3.7	0.051	QH1043	2.7	0.125	MXC67	2.2	0.226
UP8	5.0	0.063	UP3	2.5	0.127	QH1126	1.3	0.261
QH1176	3.7	0.064	SDS62	3.0	0.188	QH1075	2.0	0.262
UP2	2.3	0.073	SP7	1.6	0.207	QH1346	5.7	0.263
K82	3.7	0.076	**NA33**	**1.8**	**0.231**	SDS56	3.3	0.267
TG266	4.0	0.129	QH968	2.0	0.270	LAF1	2.8	0.296
QH37	3.2	0.174	BAL140	1.0	0.275	BAL263	4.0	0.304
SDS52	3.0	0.230	PBC159	2.0	0.380	QH1000	1.7	0.342
KKM25	4.0	0.253	QH22	1.7	0.459	QH1365	2.2	0.372
			MAN15-2	1.3	0.515	QH1306	1.0	0.411
			QH1003	1.5	0.534	QH1345	1.7	0.429
						DESA102	1.0	0.454
						DESA105	1.2	0.517
						DESA101	1.3	0.546
						EET156	3.0	0.643
						PA150	1.3	0.699
						QH326	2.3	0.789
						PBC233	2.0	0.939
						LS8	0.7	1.255
						E93(DESA)	0.8	1.498

VSD field evaluation

- **Method used**

VSD disease severity scoring was based on the intensity of natural infection of the disease on the branches (Table 2). The canopy of each tree was divided into four "quarters" (i.e. North, East, South and West). Eight to 16 branches (depending on the size of the tree canopy) were randomly selected (2 to 4 branches per quarter). VSD assessment was conducted on the terminal flushes of the labelled branches in each quarter of the canopy. Each flush was examined for infection and scored according to the scale in Table 2. The assessment was carried out at 6-months intervals over a period of 12 months or one complete epidemic cycle.

Table 2. Scoring used for evaluation of VSD severity in the field

Severity scores	Primary symptom	Other associated symptoms
0	Uninfected, healthy	Smooth bark
1	Infected leaves. Few or many, mostly symptomless	Smooth bark or with swollen lenticels
2	Infected leaves, some or most of which begin to turn chlorotic	Bark with slightly swollen lenticels
3	Infected leaves, most of which are chlorotic and necrotic, still remain attached.	Moderately rough bark
4	Infected leaves begin to abscise	Moderate to very rough bark
5	Most infected leaves abscised; apparent cessation of growth (for first flush)	Very rough bark; presence or absence of fruiting bodies
6	Near complete or complete defoliation. Dieback occurring or infected part is dead/dying	Very rough bark; with or without proliferation of auxiliary shoots; presence or absence of fruiting bodies

- **Results**

Field results cited below are considered as preliminary, because during the project only one-year data have been recorded when the plants were still relatively young. An attempt is however made to correlate the early field results with those of the *in vitro* test results.

a. Population breeding trials

The objective of the population breeding trials was to combine in the cross-progenies the following valuable traits: yield potential, VSD resistance, resistance to black pod (*Phytophthora* pod rot or Ppr) and high fat content.

Average VSD disease scores were significantly different between the crosses evaluated in the three sets of crosses carried out:

- In Set 1, DESA1 x K82, DESA1 x PA150, KA2/101 x IMC47, PBC123 x BAL244, PBC123 x IMC47, PBC123 x K82 and QH1287 x K82 showed lower VSD infection compared with the other progenies including the two resistant controls, UIT1 x NA33 and PA138 x SCA9 (Table 3).
- In Set 2, DESA1 x PBC159, KKM25 x K82 and PBC123 x UF676 exhibited low VSD disease severity compared with other genotypes and also in relation to the resistant control crosses, UIT1 x NA33 and PA138 x SCA9 (Table 4).
- In Set 3, the differences were less significant, and DESA1 x AMAZ15-15, DESA1 x T85/799, ICS95 x AMAZ15-15, KA2/101 x NA33, KKM25 x T85/799, PBC123 x NA33 did not yet exhibit any VSD infection. Most other crosses appeared to be relatively more resistant to VSD than the UIT1 x NA33 control progeny (Table 5). These preliminary results indicate that there is a good potential for VSD resistance in the crosses that were made in the population breeding programme.

Table 3. VSD disease severity assessment in the population breeding trial, Set 1

Genotype	Mean score of field evaluation [1,2]	
PBC123 x BAL244	0.14	e
PBC123 x K82	0.14	e
QH1287 x K82	0.14	e
PBC123 x IMC47	0.17	e
DESA1 x K82	0.23	de
DESA1 x PA150	0.27	cde
KA2/101 x IMC47	0.28	cde
PBC123 x PA150	0.38	bcde
KKM 25 x IMC47	0.44	bcde
PBC123 x P7	0.45	bcde
KA2/101 x P7	0.53	abcde
DESA1 x IMC47	0.54	abcde
CS95 x K82	0.56	abcde
QH1287 x PA150	0.56	abcde
QH1287 x P7	0.60	abcde
UIT1 x NA33 (C1)	0.66	abcde
KKM25 x PA150	0.71	abcde
KKM25 x P7	0.75	abcde
KKM25 x K82	0.77	abcde
ICS95 x P7	0.85	abcd
PA138 x SCA9 (C2)	0.93	abc
ICS95 x PA150	0.98	ab
KA2/101 x PA150	1.02	ab
QH1287 x IMC47	1.02	ab
ICS95 x IMC47	1.18	a
Mean	0.57	
Standard Deviation	0.31	

(1) Means in the same column followed by the same letters are not significantly different at 5% level by Tukey's Studentized Range Test (HSD).

(2) The lower the mean score, the higher the resistance in field evaluation.

Table 4. VSD disease severity assessment in the population breeding trial, Set 2

Genotype	Mean score of field evaluation [1,2]	
KKM25 x K82	0.00	e
DESA1 x PBC159	0.17	de
PBC123 x UF676	0.26	de
P138 x SCA9 (C2)	0.44	cde
ICS95 x PBC159	0.45	cde
DESA1 x UF676	0.62	bcde
PBC123 x P12	0.82	bcde
QH1287 x T85/799	0.83	bcde
DESA1 x T85/799	0.88	bcde
DESA1 x P12	1.04	bcd
ICS 95 x UF676	1.06	cde
KA 2/101 x PBC159	1.27	abc
UITI x NA33 (C1)	1.29	abc
PBC123 x PBC159	1.44	ab
ICS95 x P12	1.96	a
Mean	0.84	
Standard Deviation	0.53	

(1) Means in the same column followed by the same letters are not significantly different at 5% level by Tukey's Studentized Range Test (HSD).

(2) The lower the mean score, the higher the resistance in field evaluation.

Table 5. VSD disease severity assessment in the population breeding trial, Set 3

Genotype	Mean score of field evaluation [1,2]	
PBC123 x NA33	0.00	e
KKM25 x T85/799	0.00	e
KA2/101 x NA33	0.00	e
DESA1 x T85/799	0.00	e
DESA1 x AMAZ15-15	0.00	e
ICS95 x AMAZ15-15	0.01	e
QH1287 x NA33	0.02	e
DESA1 x PA150	0.04	e
ICS95 x NA33	0.09	de
DESA1 x PBC159	0.11	de
PBC123 x AMAZ15-15	0.23	cde
KKM25 x NA33	0.23	cde
KKM25 x AMAZ15-15	0.26	bcde
PBC123 x T85/799	0.28	bcde
PA138 x SCA9	0.29	bcde
QH1287 x T85/799	0.31	bcde
QH1287 x P12	0.58	abcd
ICS95 x T85/799	0.73	abc
UIT1 x NA33 (C1)	0.76	ab
QH1287 x AMAZ15-15	1.03	a
Mean	0.25	
Standard Deviation	0.30	

(1) Means in the same column followed by the same letters are not significantly different at 5% level by Tukey's Studentized Range Test (HSD).
(2) The lower the mean score, the higher the resistance in field evaluation.

b. International Clone Trial (ICT)

Differences between clones for the disease severity in the ICT were barely significant. Only one clone (PA7) was significantly more susceptible than the resistant local control clone PBC123 and no clone was significantly more resistant. This result suggests that observations need to be continued for more years before more significant results can be obtained.

c. Local Clone Observation Plot (LCOP)

Since the LCOP is a non-replicated trial, no statistical analysis was conducted. However, clones which did not show any VSD infection were PBC132, SDS4, LS8, DESA102, KKM25, SDS52, DESA1, SDS62, BR25, DESA103, KKM2, BAL209 and PBC123. The other clones were in the slight to moderate disease severity range.

Comparison of VSD disease severity between *in vitro* screening and field evaluation

Data on VSD disease severity in the field and *in vitro* assessment of the clones in the ICT and LCOP were compared (Tables 6 and 7). There was a slight negative correlation between the mean score of field and resistance index of *in vitro* screening of the 17 clones in the ICT and 38 clones in the LCOP. The Pearson's correlation coefficients for ICT and LCOP were -0.34 and -0.26 respectively.

Field evaluation and *in vitro* assessment of ICT clones showed that LAF1, MXC67, AMAZ15-15, PA150 and MAN15-2 were relatively more resistant to VSD for both evaluation methods as compared to clone PBC123 (Table 6). Some of the clones showed a high level of resistance in the field but were susceptible in the *in vitro* screening. However, the clones in the LCOP, LS8, DESA102, DESA105, KKM25, SDS56, PBC159 and SDS52 appeared to show low VSD scores in both *in vitro* and field evaluation to VSD (Table 7).

These results suggest that some clones that showed low VSD in the *in vitro* screening did not necessarily exhibit low scoring index in the field evaluation and *vice versa*. The difference in the resistance level between the *in vitro* and field scoring of the clones may be due to the relative young age of the trees, variation in shade intensity and tree canopy condition. Comparisons between the *in vitro* results and field evaluations carried out over more than one year are necessary before final conclusions can be drawn

Table 6. Comparison between field evaluation and *in vitro* screening of VSD disease severity assessment of the accessions in the ICT

Clone	Mean score of field evaluation [1]	Resistance index of *in vitro* screening [2]
MXC67	0.00	0.23
Mocorongo	0.04	0.02
AMAZ15-15	0.08	0.11
MAN15-2	0.17	0.52
NA33	0.17	0.04
LAF1	0.21	0.30
PA107	0.23	0.03
PA150	0.23	0.67
P12	0.29	0.05
P7A	0.31	0.10
SIAL339	0.35	0.03
PBC123 (control)	0.50	0.05
SCA6	0.75	0.02
Playa Alta 2	0.81	0.03
UF676	0.85	0.05
T85/799	0.98	0.03
P7	1.56	0.05
Mean	0.44	0.14
Standard deviation	0.42	0.19
Correlation coefficient [3]	-0.341^{ns}	

[1] The lower the mean score, the higher the resistance in field evaluation.
[2] The higher the resistance index, the higher the relative resistance (R value is not absolute). Any resistance index values better than those of the standard checks are considered as resistant.
[3] Correlation coefficient was calculated using Pearson's correlation methods on the original scale for means score field and resistance index of *in vitro* screening based on 17 clones; ns means not significant at 5% level.

Table 7. Comparison of VSD disease severity assessment between *in vitro* screening and field evaluation for the accessions in the LCOP

Clone	Mean score of field evaluation [1]	Resistance index of *in vitro* screening [2]
SDS52	0.00	0.23
DESA1	0.00	0.07
SDS62	0.00	0.19
BR 25	0.00	0.13
KKM2	0.00	0.02
PBC123	0.00	0.05
DESA102	0.00	0.45
SDS4	0.00	0.11
KKM25	0.00	0.25
LS8	0.06	1.26
P7	0.12	0.05
TG157	0.12	0.02
SDS38	0.12	0.09
QH37	0.12	0.17
SDS56	0.12	0.27
DESA105	0.12	0.52
QH1213	0.19	0.11
SDS19	0.19	0.05
FP3	0.25	0.01
QH968	0.31	0.05
PBC159	0.38	0.38
K82	0.38	0.08
QH1003	0.38	0.06
PBC236	0.56	0.03
UP8	0.62	0.06
TG149	0.69	0.03
TG146	0.75	0.04
TG137	0.75	0.07
UP3	0.81	0.13
DESA101	0.94	0.55
QH441	1.25	0.05
QH794	1.31	0.08
KKM22	1.44	0.06
TG266	1.94	0.13
QH670	2.06	0.01
KKM1	2.38	0.04
FP2	2.44	0.18
PBC179	3.25	0.04
Mean	0.63	0.16
Standard deviation	0.82	0.23
Correlation coefficient [c]	-0.26^{ns}	

[1] The lower the mean score, the higher the resistance in field evaluation.
[2] The higher the resistance index, the higher the relative resistance (R value is not absolute). Any resistance index values better than those of the standard checks are considered as resistant.
[3] Correlation coefficient was calculated using Pearson's correlation methods on the original scale for means score field and resistance index of *in vitro* screening based on 38 clones; ns means not significant at 5% level.

Conclusions

The results in the studies indicate that there was a slightly negative correlation between the level of resistance of the clones using *in vitro* screening and field evaluation. The negative correlation is expected, since high R-indexes represent higher resistance and low field scores represent also higher resistance. However, it is important that the correlations have not been significant. This would suggest that either the *in vitro* and the field evaluation method, or both methods, need to be improved. It is possible that the disease pressure in the field was still too low to obtain reliable results with only one-year observations. Long-term field evaluation would provide the real level of resistance to VSD of the clones and hybrids. The field assessments need therefore to be carried out for more years before final conclusions can be drawn on the correlation between the two methods.

Acknowledgements

The authors thank the Director-General, Malaysian Cocoa Board for the permission to present this paper and Dr Bertus Eskes, CFC/ICCO/IPGRI Project Coordinator for guidance and invaluable advices throughout the project's duration. The authors also wish to thank the Common Fund for Commodities (CFC) for funding the project.

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Evaluation of resistance to vascular streak dieback in Papua New Guinea

J. Marfu[1], Y. Efron[2] and P. Epaina[2]

[1] CCI, Stewart Research Station, PO Box 642, Madang, Papua New Guinea

[2] CCI, Tavilo Research Station, PO Box 1860, Rabaul, East New Britain, Papua New Guinea

Abstract

Vascular streak dieback (VSD) caused by the fungus *Oncobasidium theobromae* is a very damaging disease of cocoa in Papua New Guinea (PNG) and South-East Asia. In PNG it reached epidemic proportions in the 1960s, destroying plantations and preventing the establishment of new plantings. Breeding for VSD resistance is an important integral component of the PNG Cocoa and Coconut Institute breeding programme. Stewart Research Station in Madang province with high natural incidence of VSD is being used as a major site for VSD resistance breeding. A simple, inexpensive and reliable method for screening large number of genotypes at an early age in a "sick plot" was developed. Symptoms of VSD infection and the method used for assessment of VSD resistance are described. The development, maintenance and results to demonstrate the efficiency of the "sick plot" to screen large numbers of cocoa genotypes are also described.

Introduction

Vascular streak dieback (VSD) caused by the fungus *Oncobasidium theobromae* is a very damaging disease of cocoa in Papua New Guinea (PNG) and South-East Asia. In PNG it reached epidemic proportions in the 1960s, destroying plantations and preventing the establishment of new plantings.

Breeding for VSD resistance became a major breeding objective at the Department of Primary Industries (DPI). Surviving Trinitario trees from the epidemic were selected for use in the breeding programme. At the same time, the Upper Amazonian germplasm was introduced from Trinidad. In the 1980s, two Amazonian x Trinitario VSD-resistant polycross hybrids (SG1 and SG2) were developed and released for commercial planting. Consequently, VSD became economically less important. However, in some areas such as Karkar Island and Stewart Research Station (SRS) in Madang province, VSD is still very prevalent and damaging. More recently, an increase in VSD incidence was also observed in East New Britain Province, a major cocoa-producing area.

Breeding for VSD resistance is still an important integral component of the Cocoa and Coconut Institute (CCI) breeding programme. SRS, with high VSD pressure, is being used as the major screening and selection site under natural conditions. A simple, inexpensive and reliable method for screening large number of genotypes was developed in 1999. This method is being used to assess the level of VSD resistance in hybrid progenies and in an accelerated breeding scheme to develop VSD-resistant clones.

VSD infection and development of symptoms

Oncobasidium theobromae is a basidiomycete tullasnelloid fungus. So far, it has been found only in association with cocoa and no alternate hosts have been identified. The fungus is windborne. It penetrates only through the cuticle of very young leaves and then infects the xylem. Disease spread is highly dependent on moisture. Basidiospores are released at night, only after the fruiting bodies have been moistened by rain in the late afternoon or early

evening. Free water is required for spore germination and infection of leaves. Following germination and penetration, the fungus grows into the xylem. It grows down the leaf and through the leaf petiole and moves into the branch.

O. theobromae grows slowly in the branch. The first symptoms appear about 3-4 months after infection. The first infected leaf, by then located about the middle of the branch, becomes yellow with typical circular green spots. At the same time, the lenticels start to swell, giving a rough appearance to the bark of the branch. The fungus continues to grow in the xylem, filling it up and preventing water movement in the branch. Later, more leaves fall from the infected branch, the bark becomes rougher and new fruiting bodies grow out through the leaf scars. At a later stage, axillary buds often begin to grow on infected stems, but they never develop fully. When a diseased stem is split longitudinally, brown streaking of the xylem is evident. The tips of infected branches start to dry up progressively downward. Infection ends with complete death of the affected tree.

Assessment of symptoms

Symptoms are assessed on the basis of disease progression as described above over time. A 1-6 rating scale, based on visual observation, is used for individual plants as follows:

1 = Absence of VSD symptoms
2 = Appearance of yellow leaves with typical green dots, mild roughening of the bark
3 = Increased number of yellow leaves or leaf scars, increased bark roughness and appearance of fruiting bodies
4 = Advanced leaf fall, very rough bark, sprouting of multiple shoots and drying of the tips of branches
5 = Almost complete senescence of leaves in all major branches, advanced dying of branches, newly sprouting and drying shoots
6 = Complete tree death due to VSD

Clones or hybrid families are compared by a severity index (SI), i.e. the average score of all trees, and the proportion of susceptible plants (PSP), i.e. the proportion of plants having a score of 4-6.

Assessment of the International Clone Trial (ICT)

Fifty-five "international" and "local" clones were planted together in a trial at the Stewart Research Station, Madang as part of the CFC/ICCO/IPGRI project "*Cocoa Germplasm Utilization and Conservation: a Global Approach*" (Table 1). Planting was done in May 2001 in four replications of 8 trees/replication at a density of 833 trees/ha. VSD assessments started in January 2002, when the first VSD symptoms were observed. The assessment continued until the end of 2003. A wide range of variation was observed in the reaction of the clones to VSD from highly resistant (NAB11) to highly susceptible (BE10). NAB11 is a Trinitario clone collected from Karkar, an island with high VSD incidence. Most of the highly susceptible clones were introduced clones: BE10, P30, CATIE1000 and ICS1 were the most susceptible clones, but some of the local clones (K82, 38-8/2 and T45) were also among the susceptible. On the other hand, several of the introduced clones (T85/599, MAN15-2, Mocorongo and SCA6) were among the clones with the highest resistance level to VSD. The lowest average score of NAB11 was 2.1. This indicates that most of the trees showed some symptoms of VSD infection and that the probability of trees escaping infection at SRS is very low. However, the results presented in Table 1 should be considered as preliminary results since some mistakes were identified in the identity of some clones in some of the replications.

Table 1. Proportion of VSD-susceptible plants (PSP, score 4-6) and severity index (SI) of the clones tested in the International Clone Trial in SRS, Madang by December 2003

Clone	PSP (%)	SI	Clone	PSP (%)	SI	Clone	PSP (%)	SI
NAB11	0	2.1	GU225V	12	3.0	K72-7/6	50	3.9
T85/599	0	2.7	KA2-101	12	3.1	SNK64	50	4.0
KEE23	0	2.8	SIAL339	15	3.1	UF676	50	4.2
MAN15-2	0	3.0	K72-46/51	15	3.2	PA150	54	3.7
K6	4	2.2	K9	16	3.3	B22	54	3.8
K4	4	2.9	21-4-8	17	3.3	SNK413	54	4.0
KEE12	8	2.5	EET308	21	3.3	EET59	62	3.9
Mocorongo	8	2.7	IMC105	21	3.4	PA107	66	4.4
KEE43	8	2.9	K72-153/4	29	3.3	K82	66	4.4
SCA6	8	2.9	ICS95	29	3.4	38-8/2	66	4.5
IMC47	8	3.1	L14	29	3.6	MXC67	70	4.5
P7	8	3.1	EQX3360-3	29	3.7	IFC5	75	4.2
PA120	8	3.2	17-3/1	33	3.5	VENC4-4	79	4.5
LCTEEN46	8	3.2	T49	41	3.8	T45	80	4.7
66-3	8	3.2	K78-3	41	3.8	ICS1	83	4.8
K7	12	2.2	SIC5	45	4.0	CATIE1000	83	4.8
AMAZ15-15	12	2.8	36-3/1	50	3.5	P30	87	4.5
T11	12	3.0	SPEC54-1	50	3.9	BE10	90	4.5

Establishment of a "sick plot"

The favourable climatic conditions for VSD in SRS and the high incidence of the disease provided an excellent opportunity to establish a "sick plot" for screening a large number of cocoa genotypes at a relatively early age, at low cost. The establishment of the "sick plot" started with mixed planting of three known susceptible clones (K82, 38-8/2 and 17-7/4) as spreader rows at a density of 833 trees/ha (4 x 3 m). It followed, after the appearance of fruiting bodies in the spreader rows, by planting 120 seedlings each of five SG2 crosses at high density of 4.0 x 0.5 m in December 1999. The first VSD symptoms were observed in September 2000, 9 months after planting. The first assessment was done in November 2000, at which point 49 plants showed mild symptoms (Fig. 1A). Their distribution was approximately uniform throughout the plot. The number of infected plants increased exponentially between February and May (Fig. 2), reaching 100% in August 2001. By then, the distribution of susceptible plants was uniform along the rows within hybrids (Fig. 1B). The difference in the number of susceptible plants between hybrids was related to their relative resistance (Fig. 3). The cross TA33 (KEE12 x KE2-106) was the most resistant and TA17 (KEE43 x K82) was the most susceptible.

A wide range of reactions of individual plants from highly resistant to highly susceptible was found within all the crosses. This is illustrated by two short sections of six neighbouring plants each of TA16 and TA17 (Table 2). Thus, progeny 16-10 showed a susceptible reaction to VSD as early as December 2000. Progeny 17-44 behaved similarly. The development of VSD symptoms in progeny 17-39 was more gradual, starting with scores of 2 and 3 and reaching a susceptible reaction of 4 or 5 only in May. Plants 16-11, 16-14 and 17-41 were resistant, showing early minor symptoms that continued to be scored 2 or 3 throughout the period of observation. These results showed that 100% infection can be obtained in the "sick plot" and that good separation between hybrid families and individual plants within the family is achievable.

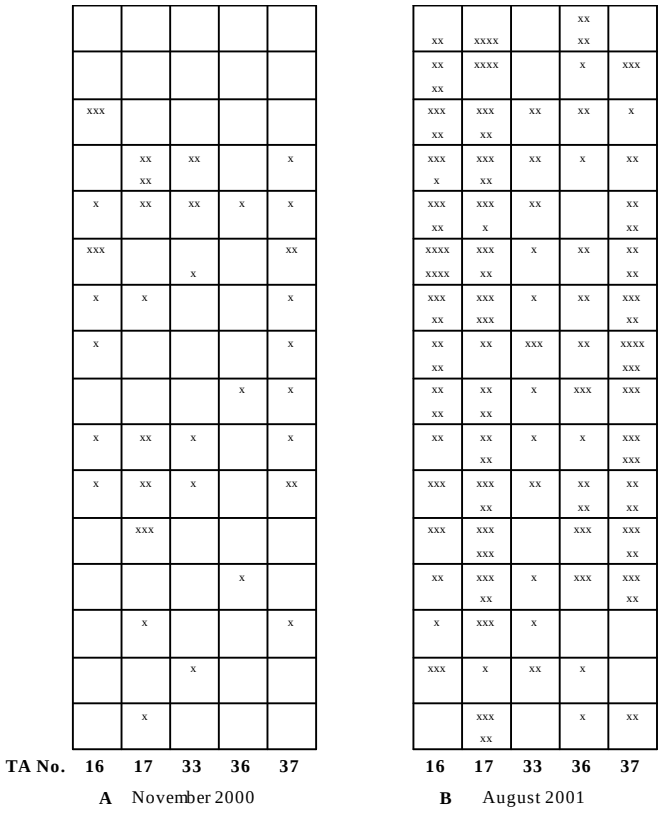

Fig. 1. Field distribution of plants showing any VSD symptoms in the first recording in November 2000 (A) and plants with severe symptoms (4 or 5) in August 2001 (B). Each square represents an area of 4 x 4 m with 8 plants in a single row. An “x” represents one plant.

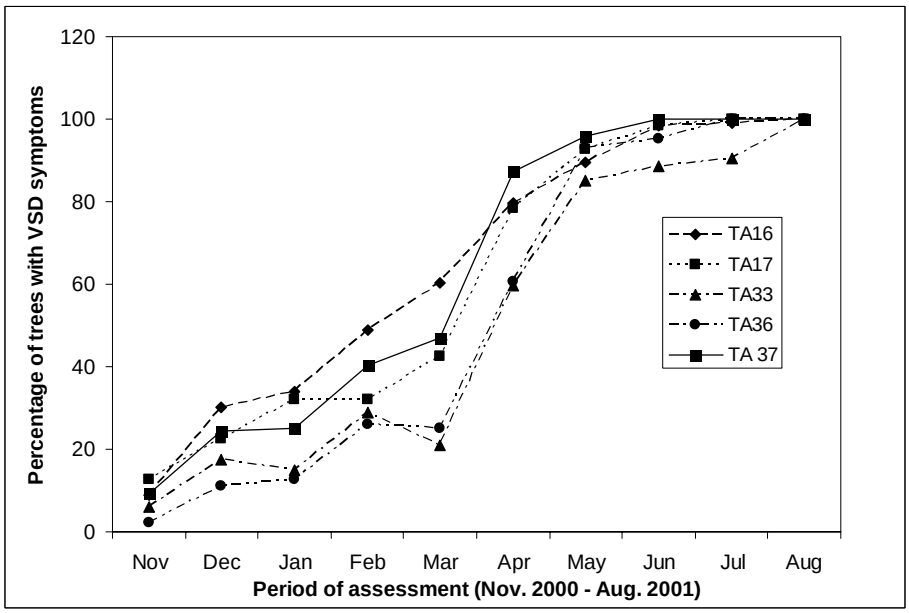

Fig. 2. Proportion (%) of cocoa trees from five Trinitario x Amazonian (TA) crosses with VSD symptoms (scores 2-5) during the period November 2000 to August 2001.

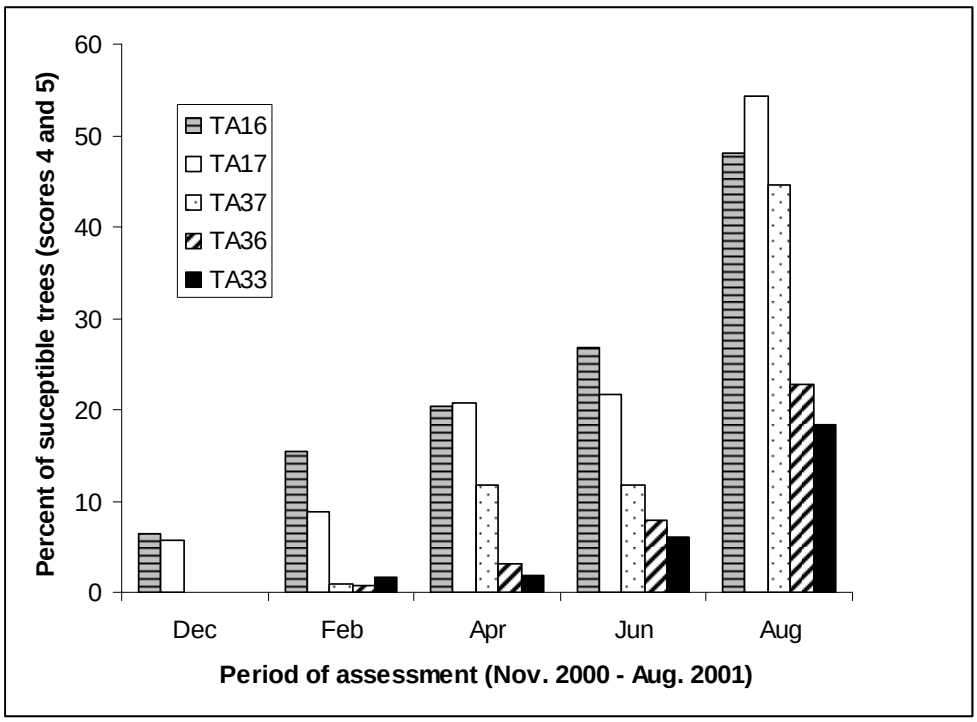

Fig. 3. Proportion (%) of cocoa trees susceptible to VSD (scores 4 and 5) in five Trinitario x Amazonian crosses (TA) at 2-months intervals from November 2000 to August 2001.

Table 2. VSD scores of neighbouring individual progenies of TA16 and TA17 showing various VSD resistance levels from November 2000 to August 2001

TA cross	Plant no.	VSD score										Comments
		Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	
16	9	1	1	1	1	1	2	2	2	2	3	Late infection, resistant
	10	1	4	4	4	5	4	4	5	5	5	Highly susceptible
	11	1	1	2	2	2	2	2	2	2	2	Early infection, stable resistance
	12	1	2	3	3	5	5	5	5	5	5	Susceptible
	13	1	2	3	3	5	5	5	5	5	5	Susceptible
	14	1	2	1	1	2	1	1	2	2	2	Highly resistant
17	39	2	1	2	2	3	3	4	5	4	5	Slow disease progression, susceptible
	40	1	1	1	1	1	2	2	2	3	4	Late infection, moderately susceptible
	41	2	2	3	3	3	2	2	2	3	3	Early infection, resistant
	42	1	1	1	1	1	2	3	3	4	4	Late infection, susceptible
	43	1	1	1	1	1	1	1	2	2	2	Late infection, resistant
	44	2	2	5	5	5	5	5	5	5	5	Highly susceptible

Assessment of eight experimental hybrids

Progenies of eight experimental hybrids (156/hybrid) of "Old" Trinitario x Upper Amazonian clones (Table 3) were interplanted in between the rows of the previously planted SG2 crosses in November 2000. A heavy load of inoculum was already present in the plot. The first symptoms appeared earlier than before to enable initial assessment in April 2002, 6 months after planting. The assessment continued at monthly intervals until September 2003.

The eight hybrids varied greatly in both the severity index and the proportion of susceptible plants (Table 3). By 2003, the proportion of susceptible trees ranged from 20.4% (KEE12 x NAB11) to 85.2% (KEE42 x B22). The crosses with RM8 and WOK1 also had a low proportion of susceptible trees (22.4% and 28.0%, respectively). Accordingly, different numbers of plants were selected as resistant plants for cloning (Table 3). These results confirm the efficiency of the "sick plot" developed at SRS, Madang to screen a large number of cocoa genotypes at a relatively early stage in a small area and at low cost.

Table 3. Proportions (%) of plants showing susceptible VSD symptoms (4-6) in progenies of eight experimental crosses between "Old" Trinitario and Upper Amazonian clones in 2002 and 2003

Cross	Origin of Trinitario parent*	Proportion (%) of susceptible plants		Trees cloned	
		2002	2003	Number	Proportion (%)
KEE12 x NAB11	Karkar, Madang	8.7	20.4	110	70.5
KEE42 x RM8	Karkar, Madang	12.1	22.4	90	57.7
KEE12 x WOK1	Karkar, Madang	18.5	28.0	79	50.6
KEE43 x T49	Tobera, ENBP	24.9	29.9	60	38.5
KEE42 x RM1	Karkar, Madang	37.7	40.1	24	15.4
KEE43 x T45	Tobera, ENBP	44.9	50.6	23	14.7
KEE43 x L14	Londip, ENBP	51.8	67.9	19	12.2
KEE42 x B72	Bali, WNBP	77.7	85.2	2	1.3
Average		34.5	43.1		

***** ENBP = East New Britain Province; WNBP = West New Britain Province

The average proportion of susceptible plants increased from 34.5% in 2002 to 43.1% in 2003. Similarly, the severity index increased progressively, but at a different rate in susceptible and resistant hybrid families (Fig. 4). However, by January 2003, the increase in the severity index had almost levelled off. This indicates that it is sufficient to follow the reaction of test materials to VSD for a period of 9-10 months.

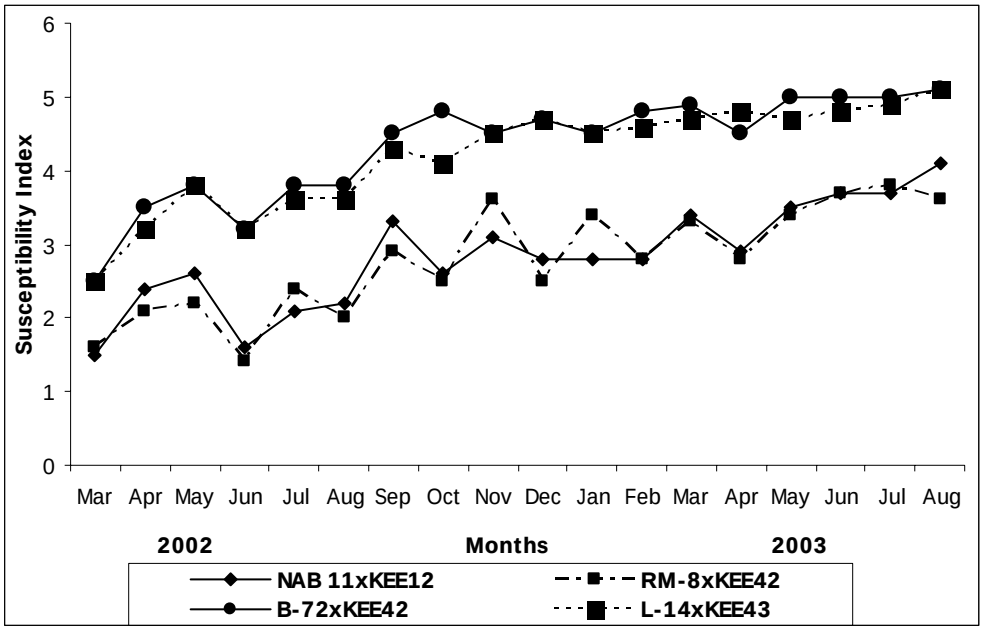

Fig. 4. VSD susceptibility index for two susceptible and two resistant "Old" Trinitario x Upper Amazonian hybrids from March 2002 to August 2003.

Maintenance of the "sick plot"

The level of VSD infection and its uniformity were sufficient to enable the identification of stable resistant genotypes of cocoa. As such, the use of natural infection at the site provided a reliable, sensitive and inexpensive method for screening a large number of cocoa genotypes at a relatively early age. Therefore, this method is particularly useful for the accelerated hybrid clone development scheme. It is recommended that the test materials be planted at a very high density of about 10 000 seedlings/ha (2.0 m x 0.5). New test materials are interplanted about 3-4 months before the removal of the previously tested materials. Planting should be done just before high rainfall is expected. In this way, the "sick plot" can be permanently maintained and screening for VSD resistance can be done continuously.

CSSV RESISTANCE STUDIES

Evaluation of resistance to cocoa swollen shoot virus (CSSV): methods, problems and selections

B. Adomako, Y. Adu-Ampomah and L.A.A. Ollennu
CRIG, PO Box 8, Tafo-Akim, Ghana

Abstract
Four cocoa groups, namely the Nanays (NA), the Parinaris (PA), the Iquitos Mixed Calabacillo (IMC) and the Trinidad introductions (T) were screened for resistance to cocoa swollen shoot virus (CSSV). Clones (budlings) were obtained from 16 Nanays, 11 Parinaris, 21 IMCs and 9 Trinidad introductions. The following F1 progenies were also created: intra-group crosses, inter-group crosses and selfed progenies. The clones/parents and the various seedling progenies were screened in the gauzehouse by inoculating the individual plants via viruliferous nymphs of mealybugs, the insect vector of the virus, and observing the plants for 3 months for virus symptoms. Whilst no significant differences were observed between the clones/parents in terms of symptom appearance, significant differences were observed in the severity of symptoms. The results indicate that resistant/tolerant genes are not confined to any one group but scattered among them. The indications are that progenies of higher resistance/tolerance can be obtained if parents of higher and varied resistance/tolerance factors are identified and used.

Introduction
A virus of great economic importance in Ghana is the cocoa swollen shoot virus (CSSV). Millions of cocoa trees have been destroyed as a result of the disease caused by the CSSV (Ollennu *et al*. 1989; Ampofo 1997). The spread of the disease has been largely unimpeded in the area of mass infection in the Eastern Region.

The only practical method to control cocoa swollen shoot virus disease (CSSVD) in Ghana has been the identification and destruction of infected trees together with their immediate, apparently symptomless, neighbouring trees (Thresh and Owusu 1986). Several million diseased trees have been removed, especially in the epidemic centre of the disease in the Eastern Region (Owusu 1983; Thresh *et al*. 1988) but adequate control of the disease has not yet been achieved.

In the earliest stages of the epidemic, a few cocoa trees remained apparently healthy in farms almost completely destroyed by the CSSVD, and it was hoped that these trees might prove immune or at least highly resistant, and so the search started for a cocoa variety that would be resistant to the CSSV (Posnette 1951).

The most satisfactory and useful way of assessing material for resistance and tolerance is in long-term field trials. However, field trials take several years and require large resources of land, labour and other inputs. It is therefore desirable that initial selection of material should rely on quicker and more amenable screening techniques. This paper reports on the use of mealybug (vector) inoculation techniques to screen cocoa genotypes for their resistance to CSSV. The mealybug inoculation method was adopted as it simulates the actual infections that occur in nature.

Materials and methods

Cocoa types

Sixteen different Nanay clones (NA), 11 Parinaris (PA), 21 Iquito Mixed Calabacillos (IMC) and 9 Trinidad introductions (T) were selected as parents on the basis of high yields and low pod rot incidence in the field. Budwood was collected from each clone and budded onto 6-month-old Amelonado rootstock to obtain 20 budlings of each clone. Pollinations were carried out to obtain the following F1 progenies: intra-group crosses, inter-group crosses (test crosses) and selfed progenies. The test crosses consisted of 27 Nanays, 14 Parinaris, 17 IMCs and 11 Ts, each crossed with three out of the four pollen parents PA7, IMC76, T16/613 and NA33, which excluded intra-group crosses in each test cross.

Screening for CSSV resistance/tolerance

The mealybug vectors of CSSV were collected from the field daily and starved for 3 days. During this period they produced nymphs. The nymphs were allowed to feed on seedlings of cocoa (A1 source plants) infected by the CSSV strain 1A (a severe strain of the virus) for 72 hours in order to acquire the virus. Ten viruliferous mealybugs were then transferred to each 6-month-old seedling/budling to be tested. The nymphs were placed in specially designed tiny cages which were then attached to each plant. The nymphs then moved out of the cages to feed on the plants. While feeding they transmit the virus into the plants. For the clones 10 budlings of each parent were tested whilst 50 seedlings per progeny were tested. The number of plants showing symptoms of CSSV disease in the first, second and third flush leaves after inoculation was recorded over a period of 3 months in the case of the seedling progenies, whilst for the budlings recordings were also made on the severity of symptoms over the 3-month period. The severity of symptoms was rated on a 0-6 scale, as follows:

0 = healthy (no symptoms)
1 = red vein banding of leaves
2 = chlorotic flecking of leaves
3 = chlorotic vein clearing and green vein banding of leaves
4 = diffused flecking of leaves
5 = fern pattern and swollen shoot
6 = dead plant

Symptoms of the disease start to appear 14 days after inoculation with red vein banding of leaves. The symptoms progress until fern patterns and swollen shoots develop and thereafter the plant starts to die.

For the seedling progenies the screening tests were repeated twice. In all over 600 crosses and 50 clones were evaluated in the laboratory for resistance to the virus. Not all the 50 clones of the four groups (populations) were used to produce the various progenies screened. Nanay and IMC clones were used in greater numbers than the Parinari and T clones in the crosses made.

Results

Clonal material

Among the clones no significant differences were observed within and between the four groups (populations) – NA, PA, IMC and T – in terms of presence of symptoms after inoculation (resistance) (data not presented) but significant differences were observed in terms of severity of symptoms (tolerance) in all the four groups (Tables 1-4). The most tolerant clones were NA335, NA750, PA81, PA88, T63/971, T61/1326, IMC22 and IMC33. The severity of the symptoms progressed with time as there were latent infections.

Table 1. Mean CSSV tolerance scores for Nanay clones

Clone	Mean CSSV score*
NA35	1.1 a
NA750	4.3 ab
NA80	5.0 ab
NA60	5.3 ab
NA744	5.7 bc
NA535	6.0 bc
NA67	6.0 bc
NA882	6.3 bc
NA2	10.0 cd
NA752	10.0 cd
NA227	11.3 de
NA876	11.3 de
NA710	13.0 def
NA244	15.0 ef
NA311	16.7 f
NA74	17.0 f
Mean	9.0
Standard error	2.10
Range	1.1-17.0
Coefficient of variation (%)	23.3

* Mean CSSV tolerance score was based on the cumulative score (severity) of all the various symptoms on each plant. Higher score indicates higher level of susceptibility. The number of scores taken from each plant depended upon the number of different symptoms that appeared.

Table 2. Mean CSSV tolerance scores for Parinari clones

Clone	Mean CSSV score*
PA81	2.3 a
PA88	4.0 ab
PA146	4.7 ab
PA6	6.0 bc
PA300	6.3 bc
PA67	6.3 bc
PA184	7.7 cd
PA134	8.3 cde
PA303	9.7 de
PA81	10.7 ef
PA181	13.0 f
Mean	7.2
Standard error	1.21
Range	2.3-13.0
Coefficient of variation (%)	16.8

* Mean CSSV tolerance score was based on the cumulative score (severity) of all the various symptoms recorded on each plant. Higher score indicates higher level of susceptibility.

Table 3. Mean CSSV tolerance scores for T clones

Clone	Mean CSSV score*
T63/971	3.0 a
T61/1326	6.0 ab
T62/2114	7.7 bc
T62/958	8.3 bc
T60/1383	9.7 cd
T87/166	9.7 cd
T63/967	10.7 cd
T61/1239	12.0 d
T65/238	12.3 d
Mean	8.8
Standard error	1.7
Range	3.0-12.3
Coefficient of variation (%)	19.3

* Mean CSSV tolerance score was based on the cumulative score (severity) of all the various symptoms recorded on each plant. Higher score indicates higher level of susceptibility.

Table 4. Mean CSSV tolerance scores for IMC clones

Clone	Mean CSSV score*
IMC22	4.0 a
IMC33	5.3 ab
IMC23	6.0 abc
IMC47	6.7 abcd
IMC11	7.0 abcd
IMC49	7.3 abcd
IMC6	8.3 bcd
IMC13	9.0 cde
IMC57	10.0 def
IMC45	11.0 efg
IMC44	13.0 fgh
IMC77	14.0 gh
IMC53	15.0 h
IMC76	15.3 h
Mean	9.3
Standard error	1.68
Range	4.0-15.3
Coefficient of variation (%)	18.1

* Mean CSSV tolerance score was based on the cumulative score (severity) of all the various symptoms recorded on each plant. Higher score indicates higher level of susceptibility.

Seedling material

In the case of the seedling progenies, significant differences were observed in the selfed Parinaris, intra-group Parinaris, inter-group Parinaris and inter-group Nanays. The most resistant among the selfed Parinari progenies were PA134, PA70 and PA52 (Table 5). For the intra-group crosses, the best selections were PA150 x PA67, PA7 x PA150 and PA52 x PA56 (Table 6). In the test crosses the best female selections for the Parinari test cross included PA134, PA51 and PA81 (Table 7) and NA235, NA153 and NA283 for the Nanay test cross (Table 8). There were no significant differences between the male parents. The most promising progenies from the test crosses included NA283 x PA7, NA179 x IMC76, NA2 x IMC76, PA134 x NA33, PA51 x IMC76, PA81 x T16/613, T79/501 x NA33, T60/887 x PA7, IMC22 x NA33 and IMC22 x T16/613 (data not presented).

For the seedling progenies, on which the experiments were repeated twice to obtain three series of results (including results from the first experiment itself), correlations between the scores for the three series of tests observed were very low (r=0.13-0.29) and not significant.

Table 5. Mean CSSV resistance score for selfed progenies of Parinari

Selfed progenies	Mean CSSV score*	
PA134	64.4	j
PA70	62.2	ij
PA52	55.6	hij
PA300	51.1	ghij
PA296	51.1	ghij
PA186	46.7	fghi
PA20	45.0	efgh
PA121	44.0	efgh
PA23	42.2	efgh
PA150	42.2	efgh
PA82	40.0	defg
PA188	40.0	defg
PA65	35.6	cdefg
PA7	31.1	bcde
PA51	24.5	abcd
PA107	24.4	abc
PA46	17.8	ab
PA146	17.8	ab
PA88	15.5	a
Mean	33.9	
Standard error	14.50	
Range	15.5-64.4	
Coefficient of variation (%)	21.4	

* Mean CSSV score was based on the percentage of plants that showed no symptoms of CSSVD after inoculation. Higher score indicates higher level of resistance.

Table 6. Mean CSSV resistance scores for Parinari intra-group crosses

Intra-group crosses	Mean CSSV score*	
PA150 x PA67	62.2	h
PA7 x PA150	53.3	gh
PA52 x PA56	51.1	fgh
PA70 x PA81	48.9	efg
PA16 x PA150	44.5	defg
PA150 x PA151	44.4	defg
PA150 x PA300	42.2	cdef
PA104 x PA107	33.3	bcd
PA51 x PA52	33.3	bcd
PA303 x PA151	33.3	bcd
PA303 x PA150	33.3	bcd
PA184 x PA107	31.1	bc
PA300 x PA134	31.1	bc
PA300 x PA184	26.7	ab
PA195 x PA296	24.4	ab
PA186 x PA184	24.4	ab
PA118 x PA184	22.2	ab
PA88 x PA121	22.2	ab
PA296 x PA7	17.8	a
Mean	35.9	
Standard error	10.91	
Range	17.8-62.2	
Coefficient of variation (%)	15.2	

* Mean CSSV score was based on the percentage of plants that showed no symptoms of CSSVD after inoculation. Higher score indicates higher level of resistance.

Table 7. Mean CSSV resistance scores for Parinari inter-group (test crosses)

Female parents	Mean CSSV score*
PA134	62.2 e
PA51	40.0 de
PA81	37.8 cde
PA121	35.6 bcd
PA70	33.4 bcd
PA118	28.9 abcd
PA16	20.0 abcd
PA300	15.6 abcd
PA107	15.5 abcd
PA184	15.5 abcd
PA150	13.3 abc
PA18	11.1 ab
PA7	6.7 a
PA52	6.7 a
Mean	24.5
Standard error	12.22
Range	6.7-62.2
Coefficient of variation (%)	49.9

Male parents	Mean CSSV score*
NA33	21.9 a
IMC76	30.5 b
T16/613	21.0 a
Mean	24.5
Standard error	3.35
Range	21.0-30.5
Coefficient of variation (%)	13.7

* Mean CSSV score was based on the percentage of plants that showed no symptoms of CSSVD after inoculation. Higher score indicates higher level of resistance.

Table 8. Mean CSSV resistance scores for Nanay inter-group (test crosses) for three series of tests

Female parents	Mean CSSV score *
NA235	66.7 g
NA153	60.0 fg
NA283	60.0 fg
NA2	57.8 efg
NA152	57.8 efg
NA244	57.8 efg
NA883	53.3 defg
NA179	53.3 defg
NA280	46.7 cdef
NA427	46.7 cdef
NA260	46.7 cdef
NA180	46.7 cdef
NA757	44.5 cdef
NA904	42.2 cdef
NA242	40.0 bcde
NA794	40.0 bcde
NA342	40.0 bcde
NA744	37.8 bcd
NA225	37.8 bcd
NA34	37.8 bcd
NA702	35.6 bcd
NA440	33.3 bc
NA682	31.1 abc
NA79	31.1 abc
NA279	28.9 abc
NA481	22.2 ab
NA227	20.0 a
Mean	42.9
Standard error	6.04
Range	20.0-66.7
Coefficient of variation (%)	14.1

Male parents	Mean CSSV score *
PA7	42.2 ab
IMC76	46.9 b
T16/613	39.5 a
Mean	42.9
Standard error	2.01
Range	39.5-46.9
Coefficient of variation (%)	4.7

* Mean CSSV score was based on the percentage of plants that showed no symptoms of CSSVD after inoculation. Higher score indicates higher level of resistance.

Comparison of CSSV tolerance/resistance scores between clonal parents, selfed, intra- and inter-group progenies

Table 9 shows the mean scores for CSSV tolerance/resistance of the clonal parents, selfed, intra- and inter-group progenies of several clones from the four populations (groups). For the Parinari population, PA134, as a clone, was susceptible; its intra-group progenies were also susceptible but its selfed and inter-group progenies were resistant to the disease. The selfed progenies of PA300 were moderately resistant but all other types of materials were susceptible to the disease. In the case of the IMC population, the clonal parent materials, the selfed and inter-group progenies were all susceptible whilst the intra-group progenies generally showed a moderate level of resistance.

Within the T population, T63/971 as a clone was highly tolerant to the disease; its selfed progeny was moderately resistant whilst its intra-group progeny was highly resistant. Although T63/967 was highly susceptible as a clone, its selfed progeny was moderately resistant whilst its intra-group progeny was the most resistant among all the populations. In the case of the Nanay population, all forms of materials from NA535 were susceptible to the disease.

Table 9. Mean CSSV resistance/tolerance scores for clones, selfed, intra- and inter-group progenies of four cocoa populations (groups)

Population/ Group	Clonal parent [1]	Selfed progenies [2]	Intra-group progenies [2]	Inter-group progenies [2]
I. Parinari				
PA300	6.3	51.1	26.7	15.6
PA134	8.3	64.4	31.1	62.2
Mean score	7.3	57.8	28.9	38.9
II. IMC				
IMC23	6.0	17.8	46.7	
IMC47	6.7	33.3	66.7	
IMC49	7.3	40.0	56.7	
IMC6	8.3	24.5	50.0	
IMC44	13.0	30.6	43.4	
IMC76	15.3	22.2	56.7	38.7
Mean score	9.4	28.1	53.3	
III. T				
T63/971	3.0	44.5	66.7	
T63/967	10.7	55.5	76.7	
Mean score	6.9	50.0	71.7	
IV. Nanay				
NA535	6.0	20.0	42.2	

[1] The clonal material was scored for tolerance to CSSV based on the cumulative score (severity) of all the various symptoms recorded on each plant. Higher score indicates higher level of susceptibility.

[2] The progenies were scored for resistance to CSSV based on the percentage of plants that showed no symptoms of the disease after inoculation. Higher score indicates higher level of resistance.

Discussion

The problem with the mealybug inoculation tests for CSSV resistance has been the difficulty in standardizing results from different series of tests (repetitions). As a result there were no significant correlations between scores from different tests. This may be due to several factors, including the condition of the mealybug vectors before inoculations. Different batches of mealybugs had to be collected from the field daily for each series of inoculations done and it is possible that the condition of the batches of mealybugs collected may vary. There is a need to intensify research into ways of rearing the mealybugs in the laboratory or gauzehouse under uniform environmental conditions, to minimize apparent variations in the behaviour of the mealybugs which may affect their feeding habits and consequently the transmission of the virus into the plants. Again the heterozygous nature of seedling material

within the same progeny may result in non-uniformity of the batches of seedlings screened. For the clones it was difficult to obtain enough budlings from some clones for the screening work. Macropropagation techniques could be employed to increase the rate of multiplication of clonal material. An alternative to the mealybug inoculation method would be the development of a manual inoculation technique that can produce sufficiently high infectious inoculum for infecting large numbers of plants to be tested.

There is also the difficulty of identifying parents with higher and varied CSSV resistance factors. Resistance to CSSV has been found to be largely polygenic (additive) (Lockwood 1981), indicating that it might be possible to increase resistance by accumulating the different resistant factors, since CSSV tolerance/resistance genes are neither confined to one particular population nor to any type of progeny but scattered among them. The characters of tolerance and resistance cannot readily be separated as they may occur in the same types of breeding material although not always to the same degree.

It appears that the CSSV resistance factors in the parents of the progenies screened are similar; thus crosses between and within them do not produce progenies with the expected levels of resistance. This similarity of resistance factors could be inferred from the origin of the parents. The PA, NA, IMC and T material may have been obtained from the same area of the Amazon Basin in South America and are thus more likely to be closely related.

Conclusion

The indications are that progenies of higher resistance/tolerance can be obtained if parents of higher and varied CSSV resistance/tolerance factors are identified by screening for resistance/tolerance in parents that are not closely related. The best parents could then be used to produce selfed, intra- and inter-group progenies for the screening tests. Final selection of the best CSSV resistant material should be based on long-term field trials because of environmental effects.

The lack of significant correlations between the scores for the three series of inoculations indicates environmental effects on CSSV symptom expression in the laboratory/gauzehouse screening tests. There is a need to improve upon the mealybug screening technique to reduce the apparent environmental effects which can have marked influence on the results obtained. This should be done with clonal materials which are genetically uniform.

Acknowledgements

The authors are grateful to the technical staff of the Plant Breeding and Pathology Divisions of the Cocoa Research Institute of Ghana.

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APPENDICES

Appendix I. Workshop Programme

Closing Workshop of the CFC/ICCO/IPGRI Project "Cocoa Germplasm Utilization and Conservation: a Global Approach" 28-31 March 2004, Reading, United Kingdom

Monday 29 March

Session 1. Opening session
Chairperson: Paul Hadley

08.30 ***Welcome words and opening of workshop*** *(Dean of the Faculty of Science of the University of Reading, representatives of IPGRI, ICCO, CFC, CPA, TWG chairperson, CWG chairperson, Session chairperson)*
09.15 ***Revisiting the Cocoa Germplasm Project rationale, objectives and finance*** *(A.B. Eskes, IPGRI/CIRAD)*
09.30 ***Outline of overall achievements of the Cocoa Germplasm Project against planned outputs*** *(J. N'Goran, TWG chairperson, and A.B. Eskes, IPGRI/CIRAD)*
10.15 ***Workshop objectives***
(T. Lass, CWG chairperson, and A.B. Eskes, IPGRI/CIRAD)

Session 2. Selection and breeding activities (results, impacts, constraints)
Chairperson: Tony Lass, Rapporteur: Olivier Sounigo

11.00 ***Breeding and selection activities in the Americas***
11.00 - Brazil *(W. Monteiro, CEPLAC)*
11.15 - Ecuador *(F. Amores, INIAP)*
11.30 - Trinidad *(K. Maharaj, MALMR)*
11.45 - Venezuela *(V. Gonzalez, FUNDACITE/INIA)*
12.00 *Discussion*
13.30 ***Breeding and selection activities in Africa***
13.30 - Cameroon *(S. Nyassé or B. Efombagn, IRAD)*
13.45 - Côte d'Ivoire *(J. N'Goran* et al., *CNRA)*
14.00 - Ghana *(Y. Adu-Ampomah, CRIG)*
14.15 - Nigeria *(K. Badaru, CRIN)*
14.30 *Discussion*
15.00 ***Breeding and selection activities in Asia***
15.00 - Malaysia *(K. Lamin, MCB)*
15.15 - PNG *(Y. Efron, CCI)*
15.30 *Discussion*

Session 3. Germplasm collection, identification and evaluation
Chairperson: Yoel Efron, Rapporteur: Kelvin Lamin

16.30 ***CFC/ICCO/IPGRI Project Collection*** *(D. Butler, CRU, and O. Sounigo, CIRAD)*
16.50 *Discussion*
17.00 ***Identification of off-types using DNA studies*** *(O. Sounigo* et al., *CIRAD)*
17.20 *Discussion*
17.30 ***Field guide for identification of widely distributed clones*** *(C. Turnbull, Univ. of Reading)*
17.50 *Discussion*
19.30 ***Discussion session on mis-labelling problems in cocoa germplasm collections***
(optional, Palmer building room P102)

Tuesday 30 March

Session 4. *Phytophthora* pod rot resistance studies and germplasm enhancement
Chairperson: David Butler, Rapporteur: Carmen Suarez

08.30 **V*alidation studies on test methods used in the project***
08.30 - Cameroon *(S. Nyassé and B. Efombagn, IRAD)*
08.45 - Côte d'Ivoire *(I. Kébé* et al.*, CNRA)*
09.00 - Trinidad *(D. Iwaro, CRU, and J.-M. Thévenin, CIRAD)*
09.15 - Brazil *(E. Luz, CEPLAC)*
09.30 **Phytophthora *pod rot resistant selections and their estimated impact on yield*** *(A.B. Eskes* et al.*, IPGRI/CIRAD)*
09.45 *Discussion and conclusions*
10.30 ***Germplasm enhancement for black pod resistance and prospects for the new project*** *(D. Iwaro, CRU)*
11.00 *Discussion and conclusions*
11.15 ***"Ring-test" on* Phytophthora *pod rot resistance using the leaf disc test***
11.15 - Studies on clone x isolate interactions *(D. Paulin and M. Ducamp, CIRAD)*
11.30 - Comparison of results obtained over project sites *(C. Cilas* et al.*, CIRAD)*
11.45 *Discussion and conclusions*
12.00 ***Molecular studies carried out on a selected progeny from PNG*** *(C. Lanaud* et al.*, CIRAD)*
12.15 *Discussion on prospects and conclusions*

Parallel Sessions 5 and 6

Parallel Session 5. Breeding and selection procedures
Chairperson: Freddy Amores, Rapporteur: Kolawole Badaru

13.30 ***Problems and solutions adopted for clone multiplication in the Project***
13.30 - Cameroon *(B. Efombagn, IRAD)*
13.40 - Nigeria *(P. Aikpokpodion, CRIN)*
13.50 - Trinidad *(D. Iwaro* et al.*, CRU)*
14.00 - Venezuela *(C. Giron* et al.*, INIA)*
14.10 *Discussion*
14.30 ***Methods proposed/used for clone multiplication at farmers' level***
14.30 - Brazil *(W. Monteiro, CEPLAC)*
14.40 - Ecuador *(F. Amores, INIAP)*
14.50 - Malaysia *(K. Lamin, MCB)*
15.00 - PNG *(P. Epaina, CCI)*
15.10 *Discussion and conclusions on procedures for clone multiplication*
16.00 ***Management and exploitation of Germplasm Project trials and observation plots*** *(A.B. Eskes, IPGRI/CIRAD)*
16.15 *Discussion*
16.30 ***Methods and prospects for accelerated hybrid clone selection*** *(Y. Efron, CCI)*
16.50 *Discussion*
17.00 ***Prospects for visual assessment of yield performance*** (*M. Tahi, CNRA*)
17.20 *Discussion*
17.30 *General discussion and conclusions*

Parallel Session 6. Resistance to VSD, CSSV and mirids (room P103)
Chairperson: Koffi N'Goran, Rapporteur: S. Nyassé

13.30 ***Evaluation of resistance to VSD, methods, problems and selections***
13.30 - PNG *(J. Marfu, CCRI)*
13.50 - Malaysia *(A. Kamil, MCB)*
14.10 *Discussion and conclusions*
14.30 ***Evaluation of resistance to CSSV: methods, problems and selections*** *(Y. Adu-Ampomah and L. Ollennu, CRIG)*
14.50 *Discussion and conclusions*
15.30 ***Resistance to cocoa mirids: methods, problems and selections***
15.30 - Cameroon *(R. Babin, CIRAD and Dibog, IRAD)*
16.00 *Discussion*
16.10 - Côte d'Ivoire *(F. N'Guessan, CNRA)*
16.40 *Discussion*
16.50 - Ghana *(B. Padi and R. K. Adu-Acheampong, CRIG)*
17.20 *Discussion*
17.30 *General discussion and conclusions*
19.30 ***Evening meeting of Technical Coordinators in preparation of presentations on the new Project*** (in Whitenights Hall cantine)
19.30 *Technical Coordinators Working Group: objectives, coordination*
20.00 *Discussions on Regional Variety Trials (Africa, America)*

Wednesday 31 March

Session 7. Resistance to witches' broom disease
Chairperson: Bob Lumsden, Rapporteur: David Iwaro

08.30 ***Witches' broom resistance screening: methods, problems and selections***
08.30 - Trinidad *(J.-M. Thévenin, CIRAD/CRU)*
08.50 - Brazil *(E. Luz, CEPLAC)*
09.10 - Ecuador (*C. Suarez, INIAP)*
09.30 - UK *(M. Shaw, Univ. of Reading)*
09.50 *General discussion, including prospects for future screening work*

Session 8. Conclusions (presented by rapporteurs) and closing session
Chairperson: Tony Lass, Rapporteur: Bertus Eskes

10.30 ***Reports on Sessions 2 to 6*** (10 min each)
11.20 *General discussion and conclusion on dissemination of results of the Cocoa Germplasm Project*
12.00 ***Closing Session*** (ICCO, CFC, IPGRI, Chairs TWG and CWG)

Appendix II. List of participants

Closing Workshop of the CFC/ICCO/IPGRI project
"Cocoa Germplasm Utilization and Conservation: a Global Approach"
28-31 March 2004, Reading, United Kingdom

Country	Surname	First name	Institution	Email address
Australia	Lambert	Smilja	Masterfoods	smilja.lambert@ap.effem.com
Brazil	Luz	Edna	CEPEC	ednadora@cepec.gov.br
	Dalva Silva	Stela	CEPEC	stela@cepec.gov.br
	Monteiro	Wilson	CEPEC	monteiro@cepec.gov.br
	Machado	Regina	Masterfoods	regina.machado@effem.com
Cameroon	Sounigo	Olivier	CIRAD	olivier.sounigo@cirad.fr
	Efombagn	Bruno	IRAD	efombagn@yahoo.fr
	Dibog	Luc	IRAD	lucdibog@yahoo.com
	Nyassé	Salomon	IRAD	nyasse@iccnet.cm
Costa Rica	Phillips	Wilbert	CATIE	wphillip@catie.ac.cr
	Johnson	Lizz	USDA/CATIE	ljohnson@catie.ac.cr
Côte d'Ivoire	Kébé	Ismael	CNRA	ibkebefr@yahoo.fr
	N'Goran	Jeanne	CNRA	jeanne_ngoran@yahoo.fr
	N'Goran	Koffi	CNRA	info.divo@cnra.ci
	N'Guessan	François	CNRA	info.divo@cnra.ci
	Tahi	Mathias	CNRA	tahi_mathias@yahoo.fr
Ecuador	Amores	Freddy	INIAP	famores_ec@yahoo.com
	Quiroz	James	INIAP	jamesq2002@yahoo.com
	Suarez	Carmen	INIAP	suarezcapello@yahoo.com
France	Cilas	Christian	CIRAD	christian.cilas@cirad.fr
	Lanaud	Claire	CIRAD	claire.lanaud@cirad.fr
	Clément	Didier	CIRAD	didier.clement@cirad.fr
	Paulin	Didier	CIRAD	didier.paulin@cirad.fr
	Cros	Emile	CIRAD	emile.cros@cirad.fr
	Ducamp	Michel	CIRAD	michel.ducamp@cirad.fr
	Petithuguenin	Philippe	CIRAD	philippe.petithuguenin@cirad.fr
	Causse	Anne	IPGRI/INIBAP	a.causse@cgiar.org
	Eskes	Bertus	IPGRI/CIRAD	b.eskes@cgiar.org
	Markham	Richard	IPGRI/INIBAP	r.markham@cgiar.org
	Vidal	Thomas	IPGRI/INIBAP	t.vidal@cgiar.org
Ghana	Abdul-Karimu	Aliyu	CRIG	
	Adomako	Boamah	CRIG	aboamah@crig.org
	Adu-Acheampong	Richard Kwame	CRIG	racheampong@crig.org
	Adu-Ampomah	Yaw	CRIG	yampomah@crig.org
	Opoku	Isaac	CRIG	iopuku@crig.org
Italy	Engels	Jan	IPGRI	j.engels@cgiar.org
Malaysia	Ahmad Kamil	Mohd Jaaffar	MCB	kamil@koko.gov.my
	Francis	Aloysius	MCB	francis@koko.gov.my
	Kelvin	Lamin	MCB	kelvin@koko.gov.my
	Sapiyah	Subali	MCB	sapiyah@koko.gov.my

Country	Surname	First name	Institution	Email address
The Netherlands	Clayton	Mark	CFC	ma_clayton@yahoo.com
	Thyssen	Marja	IAC	marja.thijssen@wur.nl
	Almekinders	Conny	WUR	conny.almekinders@wur.nl
Nigeria	Coulibaly	Nanga	COPAL	cnanga@copal-cpa.org
	Agbeniyi	Sunday	CRIN	remiagbeniyi@yahoo.com
	Aikpokpodion	Peter	CRIN	p.aikpokpodion@cgiar.org
	Badaru	Kolawole	CRIN	
	Iremiren	Gerald O.	CRIN	directorcrin@yahoo.com
	Kolesnikova-Allen	Maria	IITA	m.kolesnikova-allen@cgiar.org
	Weise	Stephan	IITA	s.weise-ibadan@cgiar.org
Peru	Garcia Carrion	Luis Fernando	UNAS	lugarc01@hotmail.com
Papua New Guinea	Efron	Yoel	CCI	yoelefron@012.net.il
	Epaina	Peter	CCI	cbreeding@ccipng.com.pg
	Marfu	Jeffrey	CCI	cbreeding@ccipng.com.pg
Trinidad	Thévenin	Jean-Marc	CIRAD	jean-marc.thevenin@cirad.fr
	Butler	David	CRU	dbutler@intrepidequipment.com
	Iwaro	David	CRU	iwaro@hotmail.com
	Maharaj	Kamaldeo	MALMR	kama1@tstt.net.tt
United Kingdom	End	Michelle	BCCCA	michelle.end@bccca.org.uk
	Lass	Tony	BCCCA	tony.lass@csplc.com
	Preece	David	BCCCA	david.preece@csplc.com
	Flood	Julie	CABI	j.flood@cabi.org
	Vos	Janny	CABI	j.vos@cabi.org
	Anga	Jean Marc	ICCO	head.econ@icco.org
	Vingerhoets	Jan	ICCO	exec.dir@icco.org
	Allaway	David	Masterfoods	david.allaway@eu.effem.com
	Gilmour	Martin	Masterfoods	martin.gilmour@eu.effem.com
	Lockwood	Rob	Masterfoods	randmlockwood@aol.com
	Binns	Helen	Un. Reading	e.binns@reading.ac.uk
	Cryer	Nick	Un. Reading	n.c.cryer@reading.ac.uk
	Davies	Alice	Un. Reading	a.e.davies@reading.ac.uk
	Daymond	Andrew	Un. Reading	a.j.daymond@reading.ac.uk
	Fenn	Megan	Un. Reading	m.g.e.fenn@reading.ac.uk
	Hadley	Paul	Un. Reading	p.hadley@reading.ac.uk
	Quainoo	Albert	Un. Reading	a.k.quainoo@reading.ac.uk
	Shaw	Michael	Un. Reading	m.w.shaw@reading.ac.uk
	Swan	Sarah	Un. Reading	s.m.swan@reading.ac.uk
	Turnbull	Chris	Un. Reading	c.j.turnbull@reading.ac.uk
	Wetten	Andrew	Un. Reading	a.c.wetten@reading.ac.uk
	Wilkinson	Mike	Un. Reading	m.j.wilkinson@reading.ac.uk
USA	Lumsden	Bob	ACRI/WCF	rdlumsden@msn.com
	Seguine	Ed	Guittard	seguine@guittard.com
	Dehnel	Roger	Masterfoods	roger.dehnel@effem.com
	Guiltinan	Mark	PennState	mjg9@psu.edu
	Motamayor	Juan-Carlos	USDA/Mars	juan.motamayor@effem.com
	Schnell	Ray	USDA	rschnell@ars-grin.gov
	Zhang	Dapeng	USDA	zhangd@ba.ars.usda.gov
Venezuela	Castillo	Angel	Fundacite/INIA	mejoramientocacao@hotmail.com
	Giron	Cirilo	Fundacite/INIA	cgironv@inia.gov.ve
	Gonzalez	Ventura	Fundacite/INIA	ventura_gonzalez@yahoo.com

Appendix III. Acronyms and abbreviations

ACD	Accelerated clone development
ACRI	American Cocoa Research Institute, USA *(now WCF)*
BCCCA	Biscuit, Cake, Chocolate and Confectionery Association, UK
BP	Black pod
CATIE	Centro Agronómico Tropical de Investigación y Enseñanza, Costa Rica
CCI	Cocoa and Coconut Institute, Papua New Guinea
CCRI	Cocoa and Coconut Research Institute, Papua New Guinea *(now CCI)*
CEPEC	Centro de Pesquisas do Cacau, Brazil
CEPLAC	Comissão Executiva do Plano da Lavoura Cacaueira, Brazil
CFC	Common Fund for Commodities, The Netherlands
CIRAD	Centre de Coopération Internationale en Recherche Agronomique pour le Développement, France
CIRAD-CP	Centre de Coopération Internationale en Recherche Agronomique pour le Développement, Département des Cultures Pérennes, France
CNRA	Centre National de Recherches Agronomiques, Côte d'Ivoire
COPAL	Cocoa Producers Alliance, Nigeria
CPB	Cocoa pod borer
CRIG	Cocoa Research Institute of Ghana
CRIN	Cocoa Research Institute of Nigeria
CRU	Cocoa Research Unit, University of the West Indies, Trinidad and Tobago
CSSV	Cocoa swollen shoot virus
FONAIAP	Fondo Nacional de Investigaciones Agropecuarias, Venezuela *(now INIA*)
FUNDACITE-Aragua	Fundación para el Desarrollo de la Ciencia y la Tecnología del Estado de Aragua, Venezuela
GCA	General combining ability
HT	Hybrid Trial
ICCO	International Cocoa Organization, UK
ICG,T	International Cocoa Genebank, Trinidad and Tobago
ICGD	International Cocoa Germplasm Database
ICT	International Clone Trial
IE	Isozyme electrophoresis
IITA	International Institute of Tropical Agriculture, Nigeria
INGENIC	International Group for Genetic Improvement of Cocoa
INIA	Instituto Nacional de Investigaciones Agropecuarias, Venezuela
INIAP	Instituto Nacional de Investigaciones Agropecuarias, Ecuador
INIBAP	International Network for the Improvement of Banana and Plantain, France
IPGRI	International Plant Genetic Resources Institute, Italy *(now Bioversity International)*
IRAD	Institut de Recherche Agricole pour le Développement, Cameroon
ITS	Internal transcribed spacer
LCOP	Local Clone Observation Plot
LCT	Local Clone Trial
MALMR	Ministry of Agriculture, Land and Marine Resources, Trinidad and Tobago
MCB	Malaysian Cocoa Board, Malaysia
PCR	Polymerase chain reaction
Ppr	*Phytophthora* pod rot
QTL	Quantitative trait locus
RAPD	Random amplified polymorphic DNA
RRS	Reciprocal recurrent selection
SEM	Standard error of the mean
SSR	Simple sequence repeats
UWI	University of the West Indies, Trinidad and Tobago
VSD	Vascular streak dieback
WB	Witches' broom
WCF	World Cocoa Foundation, USA

INDEX OF AUTHORS

ISBN-13: 978-92-9043-734-5
ISBN-10: 92-9043-734-0